XXII Congreso Latinoamericano de Bioquímica Clínica
Punta del Este – Septiembre 20, 2017
(Auditórium Principal – 11:50 – 12:40)

Resistance to polymyxins: genetic bases, epidemiology, and detection issues

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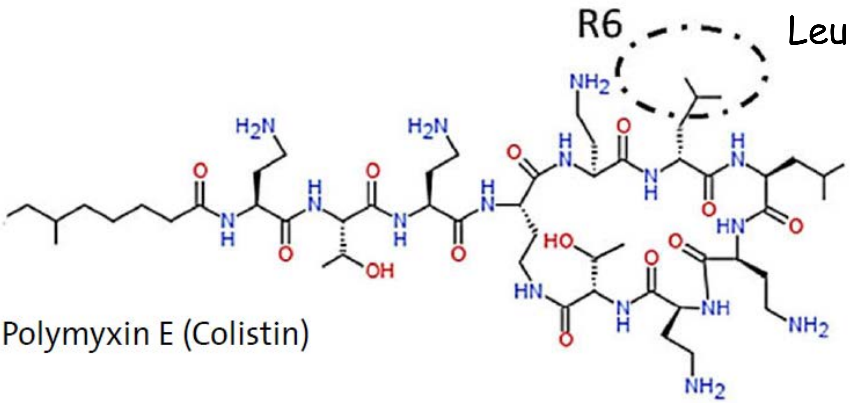
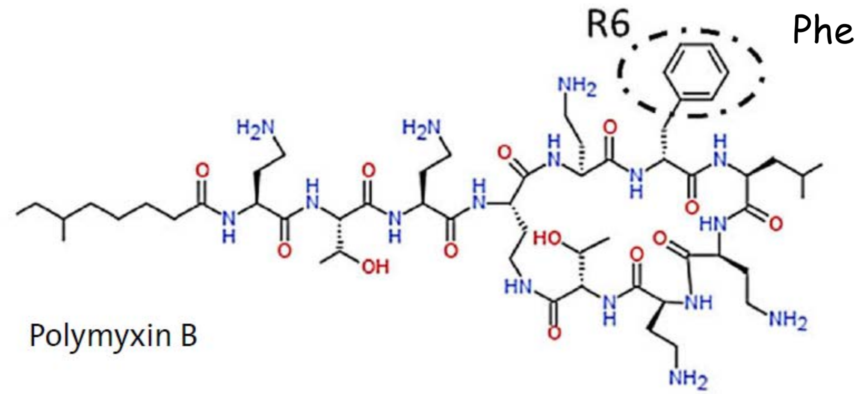
University of Bern, Switzerland

Polymyxins

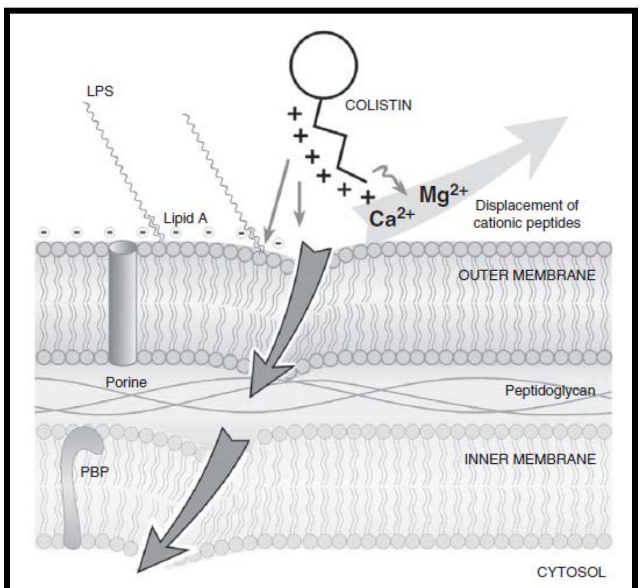


Polymyxin E (Colistin)



Polymyxin B

1947 from *Paenibacillus polymyxa* subsp. *colistinus*
1959: FDA approved the prodrug (colistimethate, CMS) for parenteral use



Narrow spectrum against Gram-negatives

Most *Enterobacteriaceae*: *E. coli*, *Enterobacter* spp. *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., and *Shigella* spp.

Nonfermenters: *A. baumannii*, *P.* aeruginosa, *Steno. maltophilia*

Naturally resistant: *Proteus spp., M. morganii, Providencia spp., S. marcescens, Ps. mallei, B. cepacia, Brucella, Legionella, Campylobacter, V. cholerae*

Klebsiella pneumoniae

	Antibiotics tested	Wildtype (WT)	MDR Multidrug-resistant	XDR Extensively drug-resistant	PDR Pandrug-resistant
Beta-Lactams Penicillins	Ampicillin	R	R	R	R
Penicillins "protected"	Amoxicillin-clav.	S	I/R	R	R
	Piperacillin-tazob.	S	I/R	R	R
Cephalosporins Ig	Cefoxitin	S	R	R	R
	Cephalotin	S	R	R	R
IIg	Cefpodoxime	S	R	R	R
IIIg	Ceftriaxone	S	R	R	R
	Cefotaxime	S	R	R	R
	Ceftazidime	S	R	R	R
IVg	Cefepime	S	S/R	R	R
Monobactams	Aztreonam	S	R	R	R
Carbapenems	Imipenem	S	S	R	R
	Meropenem	S	S	R	R
Aminoglycosides	Gentamicin	S	S/R	R	R
	Tobramycin	S	S/R	R	R
	Amikacin	S	S	R	R
Quinolones	Ciprofloxacin	S	R	R	R
Tetracyclines	Doxycycline	S	R	R	R
Miscellaneous	Trimethoprim/sulfa.	S	R	R	R
"LAST OPTIONS"	Fosfomycin	S	S	S/R	R
	Tigecycline	S	S	S/R	R
	Colistin	S	S	S/R	R

ESBLs

Carbapenemases

Frequently co-associated with ESBLs and carbapenemases

Summary of the latest data on antibiotic consumption in the European Union

ESAC-Net surveillance data
November 2016

Table 5. Trends in consumption of polymyxins in the hospital sector, EU/EEA countries, 2011–2015 (expressed as DDD per 1 000 inhabitants and per day)

Country	2011	2012	2013	2014	2015	Trends in consumption of polymyxins, 2011–2015	Average annual change 2011–2015	Statistically significant trend
Finland (b)	0	0	0	0	0		<0.001	
Lithuania (a)		0	0	0	0			N/A
Latvia	0	0.003	0.002	0.001	<0.001		<0.001	
Norway	0.0004	0.0006	0.0006	0.0006	0.0007		<0.001	>
Sweden	0.001	0.001	0.001	0.001	0.001		<0.001	
Netherlands	0.003	0.002	0.003	0.002	0.003		<0.001	
Estonia	<0.001	0.002	0	0.002	0.003		0.001	
Bulgaria	0	0	0	0.002	0.004		0.001	>
Luxembourg	0.005	0.005	0.006	0.003	0.005		<0.001	
Denmark	0.002	0.002	0.001	0.003	0.005		0.001	>
Slovenia	0.002	0.003	0.003	0.005	0.005		0.001	
United Kingdom (a)			0.005	0.006	0.006			N/A
Belgium	0.009	0.006	0.008	0.008	0.007		<0.001	
France	0.008	0.008	0.008	0.008	0.007		<0.001	
Ireland	0.014	0.015	0.015	0.013	0.008		-0.001	
Hungary	0.004	0.005	0.006	0.007	0.008		0.001	>
EU/EEA	**0.011**	**0.014**	**0.012**	**0.012**	**0.015**		**<0.001**	
Croatia	0.010	0.029	0.003	0.019	0.018		0.001	
Malta	0.004	0.002	0.006	0.011	0.020		0.004	>
Poland (a)				0.001	0.020			N/A
Portugal (c)	0.018	0.019	0.020	0.019	0.022		0.001	
Cyprus	0.014*	0.013*	0.023*	0.023*	0.023*		0.003	
Slovakia (a)		0.020	0.023	0.025	0.024			N/A
Italy	0.011	0.019	0.023	0.025	0.027		0.004	>
Romania	0.019*	0.020*	0.026*	0.027*	0.034*		0.004	>
Greece	0.078	0.085	0.084	0.095	0.095		0.004	>

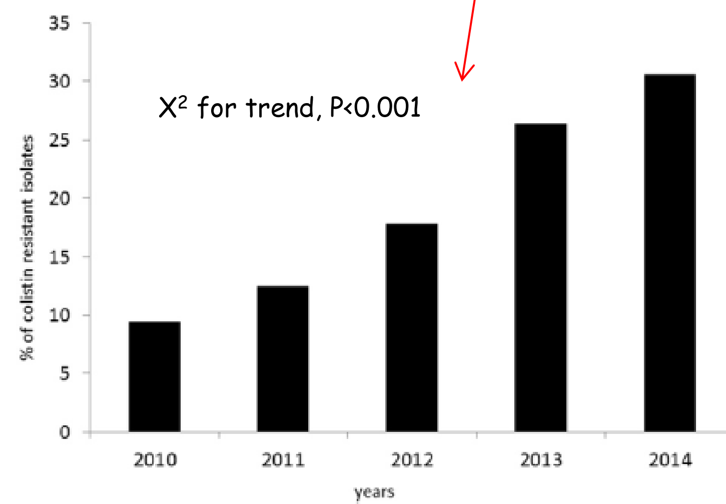
Risk factors for bloodstream infections due to colistin-resistant KPC-producing *Klebsiella pneumoniae*: results from a multicenter case–control–control study Giacobbe DR et al., CMI, 2015

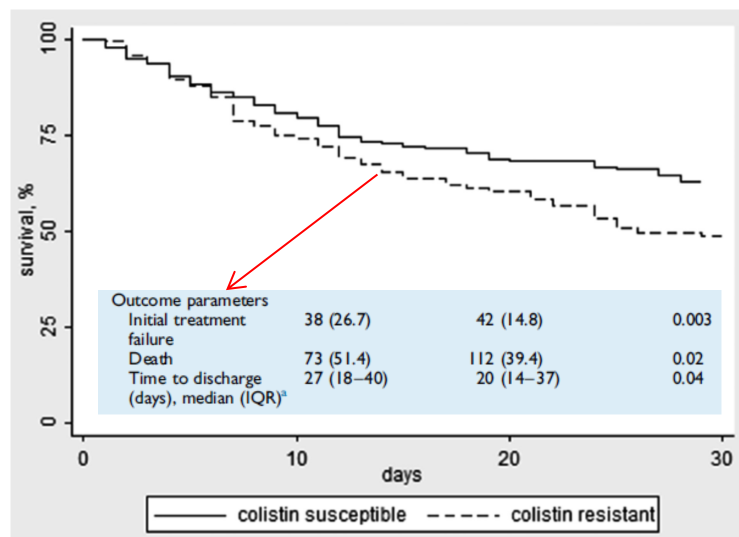
Variable	Case group (*n* = 142)	Control group B (*n* = 284)	OR (95% CI)	p
Age (years), median (IQR)	66 (54–74)	67 (55–77)	—	0.23
Male sex	89 (62.7)	180 (63.4)	0.97 (0.63–1.51)	0.89
Comorbidities				
COPD	14 (9.9)	41 (14.4)	0.65 (0.31–1.27)	0.18
Biliary devices	1 (0.7)	7 (2.5)	0.28 (0.01–2.22)	0.21
Cardiovascular diseases	53 (37.3)	120 (42.2)	0.81 (0.52–1.25)	0.33
Cerebrovascular diseases and dementia	15 (10.6)	35 (12.3)	0.84 (0.41–1.65)	0.59
Solid organ cancer	26 (18.3)	50 (17.6)	1.05 (0.59–1.82)	0.86
Hematologic cancer	26 (18.3)	43 (15.1)	1.26 (0.70–2.21)	0.40
Diabetes mellitus	30 (21.1)	71 (25.0)	0.80 (0.48–1.33)	0.37
Splenectomy	2 (1.4)	4 (1.4)	1 (0.09–7.07)	1.00
Chronic renal failure	23 (16.2)	54 (19.0)	0.82 (0.46–1.44)	0.48
Liver disease	13 (9.1)	27 (9.5)	0.96 (0.44–2.00)	0.91
HIV infection	3 (2.1)	13 (4.6)	0.45 (0.08–1.68)	0.21
Solid organ transplantation	13 (9.1)	21 (7.4)	1.26 (0.56–2.74)	0.53
Charlson score ≥3	41 (28.9)	39 (13.7)	2.25 (1.50–4.31)	<0.001
History				
Previous hospitalization	94 (66.2)	.	1.24 (0.80–1.93)	0.32
≥1	79 (55.6)	121 (42.6)	1.69 (1.10–2.59)	0.01
≥2	48 (33.8)	60 (21.1)	1.91 (1.18–3.06)	0.005
≥3	30 (21.1)	36 (12.7)	1.84 (1.04–3.25)	0.02
≥4	15 (10.6)	26 (9.1)	1.17 (0.56–2.39)	0.64
≥5	12 (8.4)	19 (6.7)	1.29 (0.55–2.89)	0.51
Outpatient follow-up	32 (22.5)	49 (17.2)	1.39 (0.81–2.36)	0.19
Admission from another healthcare facility	3 (2.1)	19 (6.7)	0.30 (0.06–1.05)	0.04
Previous ICU admission	38 (26.8)	54 (19.0)	1.56 (0.94–2.57)	0.07
Intravenous home therapy	2 (1.4)	2 (0.7)	2.01 (0.14–28.00)	0.48
Previous urinary tract infection	19 (13.4)	42 (14.8)	0.89 (0.47–1.64)	0.69
Recent bacterial infections	67 (47.2)	93 (32.7)	1.83 (1.19–2.83)	0.004
Previous MRSA isolation	5 (3.5)	13 (4.6)	0.76 (0.21–2.33)	0.61
Previous ESBL-producer isolation	12 (8.4)	20 (7.0)	1.22 (0.52–2.71)	0.60
Previous VRE isolation	1 (0.7)	3 (1.1)	0.66 (0.01–8.36)	0.72
Previous KPC-Kp colonization	51 (35.9)	49 (17.2)	2.69 (1.64–4.37)	<0.001
Neutropenia	16 (11.3)	37 (13.0)	0.85 (0.42–1.63)	0.60
CVC	97 (68.3)	175 (61.6)	1.34 (0.86–2.11)	0.17
Nasogastric tube	42 (29.6)	61 (21.5)	1.53 (0.94–2.48)	0.06
Surgical drainage	21 (14.8)	51 (18.0)	0.79 (0.43–1.41)	0.41
Urinary catheter	85 (59.9)	159 (56.0)	1.17 (0.76–1.80)	0.45
Endoscopy	14 (9.9)	30 (10.6)	0.93 (0.44–1.88)	0.82
Mechanical ventilation	48 (33.8)	98 (34.5)	0.97 (0.62–1.51)	0.88
Dialysis	21 (14.8)	41 (14.4)	1.03 (0.55–1.87)	0.92
Total parenteral nutrition	31 (21.8)	83 (29.2)	0.68 (0.41–1.11)	0.10
Immunosuppressive therapy	18 (12.7)	25 (8.8)	1.50 (0.74–2.99)	0.21
Corticosteroid therapy	22 (15.5)	46 (16.2)	0.95 (0.52–1.70)	0.85
Chemotherapy/radiotherapy	18 (12.7)	39 (13.7)	0.91 (0.47–1.71)	0.76
PEG	4 (2.8)	8 (2.8)	1.00 (0.22–3.81)	1.00
Bedridden	30 (21.1)	51 (18.0)	1.22 (0.71–2.08)	0.43
Previous surgery	63 (44.4)	120 (42.2)	1.09 (0.71–1.67)	0.68
Recent antibiotic therapy				
In general	119 (83.8)	240 (84.5)	0.95 (0.53–1.73)	0.85
By classes				
Aminoglycosides	15 (10.6)	30 (10.6)	1.00 (0.48–2.00)	1.00
β-Lactam-β-lactamase inhibitor	53 (37.3)	150 (52.8)	0.53 (0.34–0.82)	0.002
Fluoroquinolones	47 (33.1)	95 (33.4)	0.98 (0.62–1.54)	0.94
Oxyiminocephalosporins	19 (13.4)	38 (13.4)	1.00 (0.52–1.87)	1.00
Carbapenems	45 (31.7)	92 (32.4)	0.97 (0.61–1.52)	0.88
Glycopeptides	39 (27.5)	62 (21.8)	1.35 (0.83–2.21)	0.20
Colistin	40 (28.2)	15 (5.3)	7.03 (3.60–14.25)	<0.001
Other	49 (34.5)	80 (28.2)	1.34 (0.85–2.11)	0.18

Six Italian hospitals, Jan 2010 – June 2014

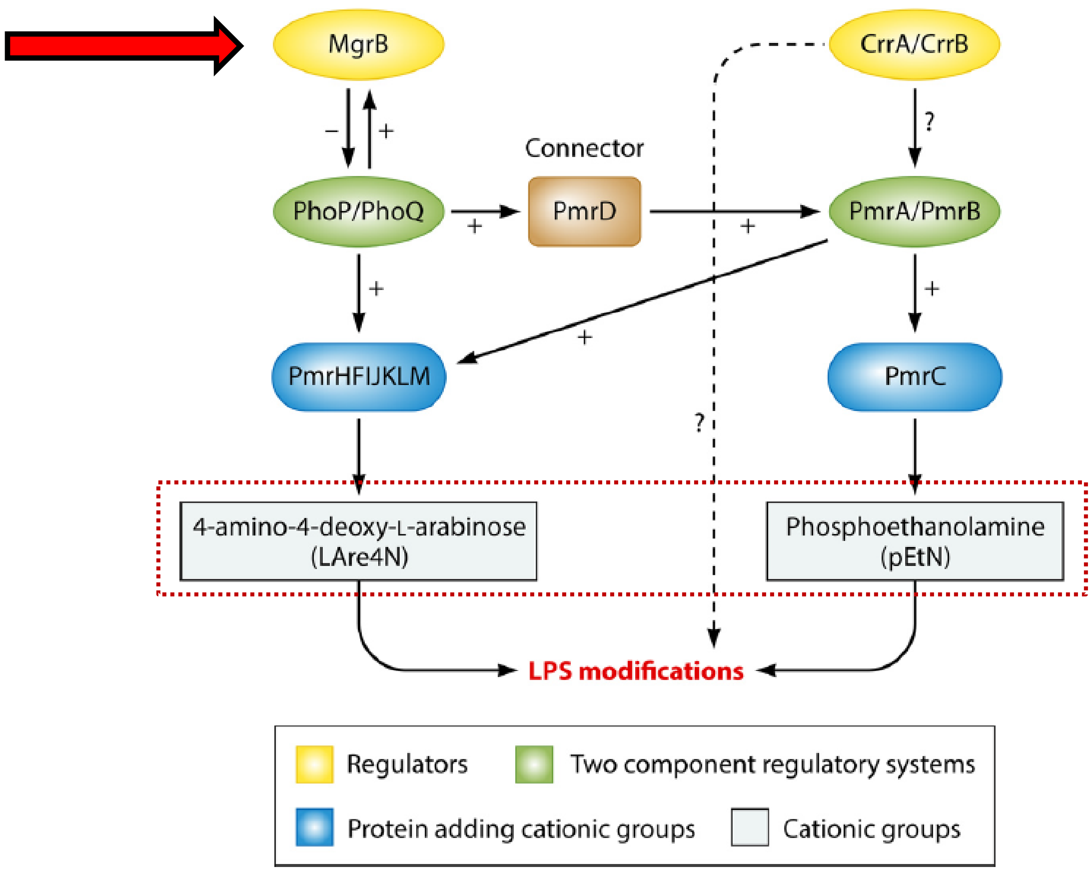
Monomicrobial BSI due to KPC-*K. pneumoniae*

Colistin-S (control) *vs.* Colistin-R (case)





Acquired Resistance: "Chromosomal First"



CATIONIC GROUPS
Naturally R species express
arnBCADTEF operon and/or *eptB* gene

A large panel of genes and operons are involved in qualitative modification of LPS

(Poirel L et al., Clinical Microbiol Review, April 2017)

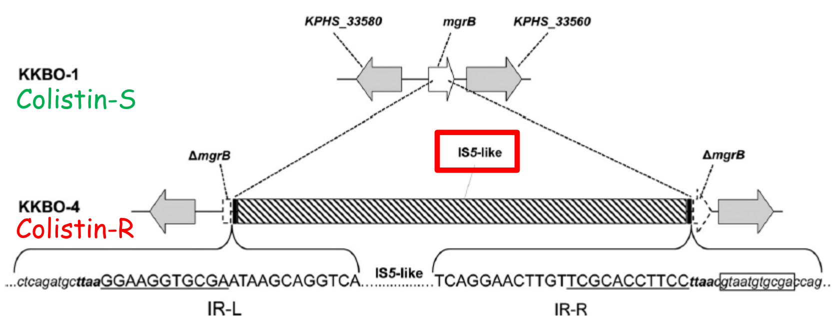
In Vivo Emergence of Colistin Resistance in *Klebsiella pneumoniae* Producing KPC-Type Carbapenemases Mediated by Insertional Inactivation of the PhoQ/PhoP *mgrB* Regulator

November 2013 Volume 57 Number 11 Antimicrobial Agents and Chemotherapy p. 5521–5526

Antonio Cannatelli,[a] Marco Maria D'Andrea,[a] Tommaso Giani,[a] Vincenzo Di Pilato,[a] Fabio Arena,[a] Simone Ambretti,[b] Paolo Gaibani,[c] Gian Maria Rossolini,[a,d,e]

ICU-patient – Italy (Bologna)

- KKBO-1: KPC-Kp Colistin-S in blood
    - Colistin aerosolized, 25 days
    - Colistin i.v., 14 days
- 30 days later
  KKBO-4: KPC-Kp Colistin-R in blood
- Identical strains (=PFGE and ST258)



Also confirmed *in vitro*

- KKBO-1 on agar plates with colistin [8 mg/L]
- Colistin-R mutants with 7 x 10^{-7} frequency
    - 60% had insertion of IS*5*-like
    - No fitness cost (same authors; *AAC*, 2015)

The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*

J Antimicrob Chemother 2015; **70**: 75–80

Laurent Poirel[1,2], Aurélie Jayol[2], Séverine Bontron[1], Maria-Virginia Villegas[3], Melda Ozdamar[4], Salih Türkoglu[4] and Patrice Nordmann[1,2,5*]

47 Col-R KPC-Kp (mostly ST258)

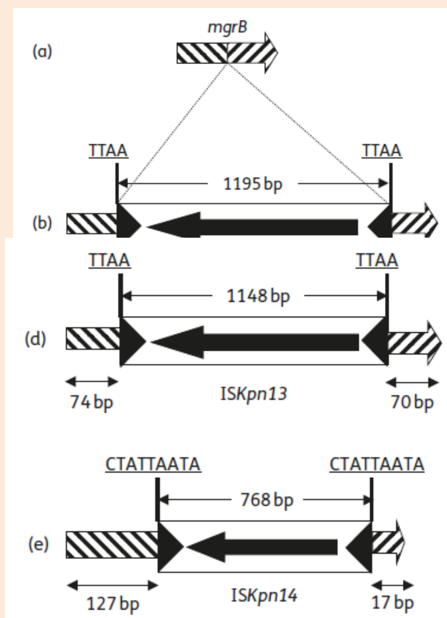
12 had mutated *mgrB*

Isolate	Country of isolation	*mgrB* sequence	Insertion site between
T1a	Turkey	wild-type	—
T1b	Turkey	truncated by IS5-like	+74 and +75
11I8	France	truncated by IS5-like	+74 and +75
20C9	France	truncated by IS5-like	+74 and +75
C9	Colombia	truncated by IS5-like	+74 and +75
C10	Colombia	truncated by IS5-like	+74 and +75
C21	Colombia	truncated by IS*Kpn13*	+74 and +75
C22	Colombia	truncated by IS*Kpn14*	+127 and +128
C1	Colombia	truncated by IS*10R*	−27 and −26
C2	Colombia	truncated by IS*10R*	−27 and −26
C11	Colombia	truncated protein (29 aa)	—
15I5	France	truncated protein (29 aa)	—
Sa	France	truncated protein (27 aa)	—

More IS inserted in *mgrB*

IS in the promoter of *mgrB*

Stop codon *mgrB*



MgrB Inactivation Is a Common Mechanism of Colistin Resistance in KPC-Producing *Klebsiella pneumoniae* of Clinical Origin

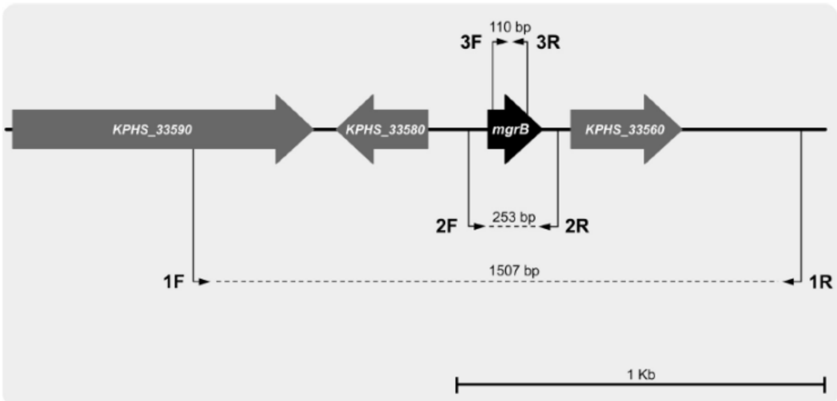
Antimicrobial Agents and Chemotherapy p. 5696–5703 October 2014 Volume 58 Number 10

Antonio Cannatelli,[a] Tommaso Giani,[a] Marco Maria D'Andrea,[a] Vincenzo Di Pilato,[a] Fabio Arena,[a] Viola Conte,[a] Kyriaki Tryfinopoulou,[b] the COLGRIT Study Group, Alkiviadis Vatopoulos,[b,c] Gian Maria Rossolini[a,d,e]

Italy and Greece, 2010-2012

66 Col-R KPC-KP

Mostly, ST258 and ST512



Transcriptional analysis (qPCR)

mgrB lesion[b]	*pmrK* expression (mean ± SD)	*pmrK* expression after complementation (mean ± SD)	*phoQ* expression (mean ± SD)	*phoQ* expression after complementation (mean ± SD)
WT Col-S	1	1.24 ± 0.51	1	0.93 ± 0.18
Insertional inactivation, IS5-like element at nt 75 (RW)	6.19 ± 0.19	1.34 ± 0.13	3.56 ± 0.15	0.92 ± 0.17
Insertional inactivation, IS5-like element at nt 126 (FW)	7.97 ± 0.12	1.81 ± 0.11	4.42 ± 0.25	1.46 ± 0.35
Insertional inactivation, IS*1F*-like element at nt 105 (FW)	8.54 ± 0.09	2.04 ± 0.23	8.81 ± 0.44	1.11 ± 0.14
t71a (L24H)	3.25 ± 0.23	1.87 ± 0.37	2.98 ± 0.77	1.24 ± 0.26
Δ18/27 (frameshift and premature termination)	5.15 ± 0.04	1.59 ± 0.34	7.23 ± 0.30	2.18 ± 0.45
g109a (G37S)	6.61 ± 0.07	1.97 ± 0.14	6.65 ± 0.21	0.48 ± 0.34
a7t (nonsense, premature termination)	5.44 ± 0.11	1.82 ± 0.22	4.22 ± 0.29	2.18 ± 0.33
Δg47 (frameshift and premature termination)	10.39 ± 1.12	1.10 ± 0.04	11.45 ± 1.23	1.08 ± 0.45
g83a (C28Y)	9.51 ± 0.32	1.48 ± 0.20	14.02 ± 1.17	3.71 ± 0.75
Δ109/119 (frameshift and premature termination)	5.37 ± 0.15	1.69 ± 0.17	9.61 ± 0.55	1.59 ± 0.15
Insertional inactivation, IS*Kpn14* at nt 124 (FW)	5.97 ± 0.18	1.99 ± 0.18	4.72 ± 0.35	1.20 ± 0.39
Δ*mgrB* (from −400 to +599)	7.86 ± 0.87	2.08 ± 0.34	4.08 ± 0.23	0.97 ± 0.11
Δ*mgrB* locus	3.26 ± 0.07	0.78 ± 0.24	7.54 ± 0.54	1.75 ± 0.65
Δ*mgrB* locus	4.21 ± 0.13	1.77 ± 0.13	5.05 ± 0.05	1.02 ± 0.34
Δ*mgrB* locus	5.75 ± 0.52	1.08 ± 0.36	4.51 ± 0.39	0.77 ± 0.43

mgrB status:

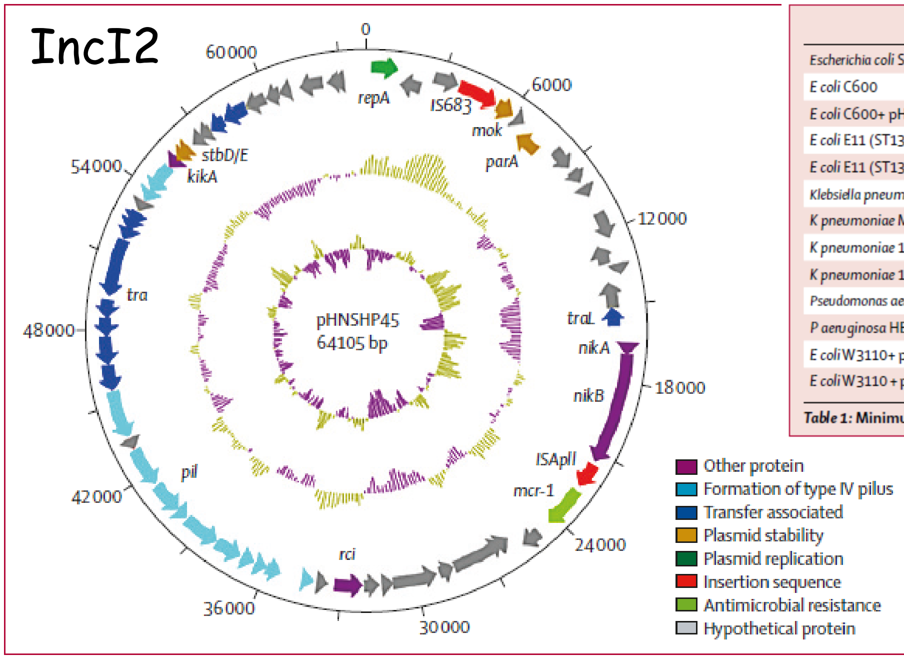
- **41%: WT**
- **27%: Insertion of IS*5*-like at nt 75**
- 8%: g109a (G37S)
- 5%: Δ*mgrB* locus? No amplification with 1F/R
- 3%: Insertion of IS*Kpn14* at nt 124
- Δ18/27 (frameshift and premature termination)
- a7t (nonsense, premature termination)
- Δg47 (frameshift and premature termination)
- Δ109/119 (frameshift and premature termination)
- Insertion of IS*1F*-like at nt 105
- Δ*mgrB* (from -400 to +599)
- t71a (L24H)
- g83a (C28Y)

All affect *pmrHFIJKLM* operon, thus LPS

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen*

Lancet Infect. Dis. 2015 November 18



	Origin	Polymyxin E (colistin)	Polymyxin B
Escherichia coli SHP45 (*mcr-1*)	Pig	8·0	4·0
E coli C600	..	0·5	0·5
E coli C600+ pHNSHP45 *mcr-1*)	Transconjugant	8·0	4·0
E coli E11 (ST131, KPC-2-producer)	Human	0·5	0·5
E coli E11 (ST131, KPC-2-producer) + pHNSHP45 (*mcr-1*)	Transformant	4·0	2·0
Klebsiella pneumoniae MPC11	Human	0·5	0·5
K pneumoniae MPC11 + pHNSHP45 (*mcr-1*)	Transformant	8·0	4·0
K pneumoniae 1202 (ST11, KPC-2-producer)	Human	0·5	0·5
K pneumoniae 1202 (ST11, KPC-2-producer) + pHNSHP45 (*mcr-1*)	Transformant	4·0	4·0
Pseudomonas aeruginosa HE26	Human	0·5	0·5
P aeruginosa HE26 + pHNSHP45(*mcr-1*)	Transformant	8·0	4·0
E coli W3110+ pUC18	Laboratory strain	0·5	0·5
E coli W3110 + pUC18-*mcr-1*	Transformant	2·0	2·0

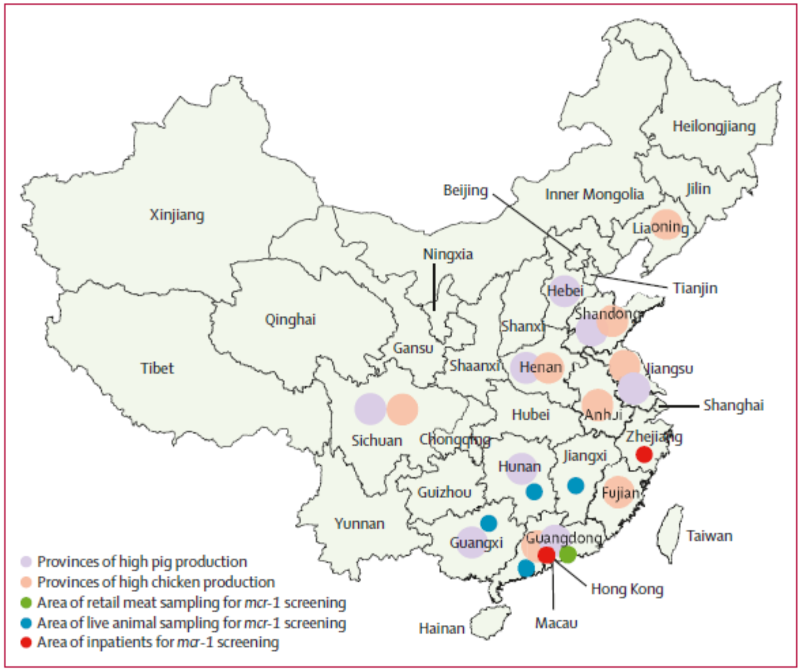
Table 1: Minimum inhibitory concentration (mg/L) for parental strain, transformants, and transconjugant

Highly conjugative with *E. coli*
(frequency of 10^1-10^3 cells per recipient)

mcr-1 encodes for a Phosphoethanolamine transferase enzyme

	Year	Positive isolates (%)/number of isolates
Escherichia coli		
Pigs at slaughter	All	166 (20·6%)/804
Pigs at slaughter	2012	31 (14·4%)/216
Pigs at slaughter	2013	68 (25·4%)/268
Pigs at slaughter	2014	67 (20·9%)/320
Retail meat	All	78 (14·9%)/523
Chicken	2011	10 (4·9%)/206
Pork	2011	3 (6·3%)/48
Chicken	2013	4 (25·0%)/16
Pork	2013	11 (22·9%)/48
Chicken	2014	21 (28·0%)/75
Pork	2014	29 (22·3%)/130
Inpatient	2014	13 (1·4%)/902
Klebsiella pneumoniae		
Inpatient	2014	3 (0·7%)/420

Table 2: Prevalence of colistin resistance gene *mcr*-1 by origin



GERMINATION *A Blog by Maryn McKenna*

Apocalypse Pig: The Last Antibiotic Begins to Fail

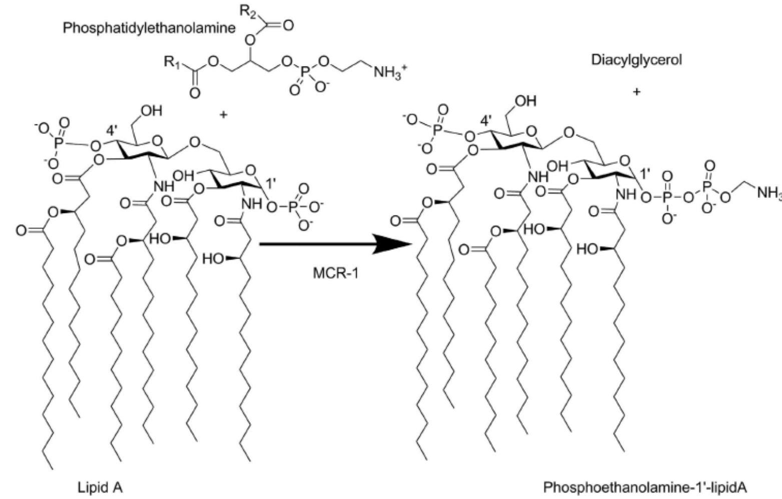
POSTED SAT, 11/21/2015

NATIONAL GEOGRAPHIC

Sales (in kg)	2006	2007	2008	2009	2010	2011	2012	2013
Use of antibiotics in Food Producing Animals - SWITZERLAND								
Sulfonamides	27,025	29,086	29,088	27,231	25,672	23,118	21,556	18,942
Penicillins	12,632	12,630	13,300	12,650	12,996	13,277	12,803	12,645
Tetracyclines	14,992	16,664	16,704	15,546	14,746	13,731	12,038	11,626
Macrolides + lincosamides	3,667	4,081	4,338	4,063	3,864	3,508	3,326	3,125
Aminoglycosides	3,692	3,688	3,688	3,549	3,215	3,317	3,199	3,115
Trimethoprim	2,079	2,013	1,854	1,749	1,702	1,548	1,368	1,148
Polymyxins	1,829	1,666	1,577	1,543	1,489	1,454	1,057	854
Fluoroquinolones	318	360	408	403	388	371	335	384
Cephalosporins	131	152	169	203	237	249	237	228
Amphenicols								183
Others (*)	122	295	191	211	245	568	413	274
Total	**66,487**	**70,633**	**71,316**	**67,147**	**64,554**	**61,140**	**56,332**	**52,250**

Insights into the Mechanistic Basis of Plasmid-Mediated Colistin Resistance from Crystal Structures of the Catalytic Domain of MCR-1

Hinchliffe P et al., Scientific Reports, Jan 2017

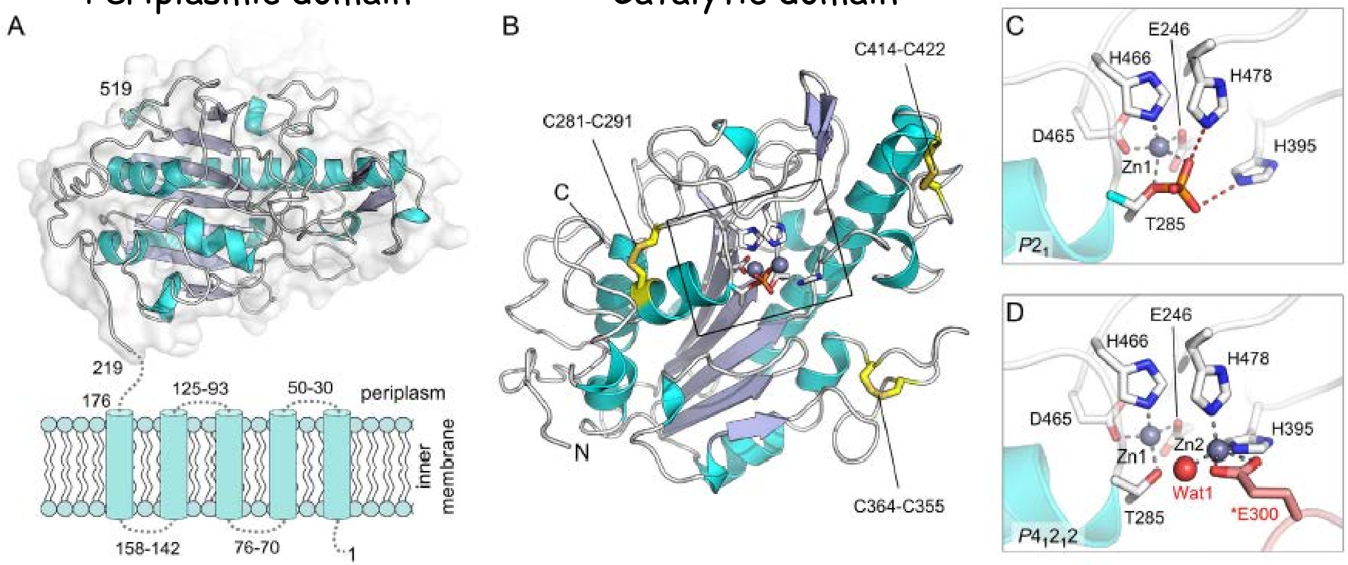


MCR-1 catalyzes transfer of PEA onto glucosamine saccharide of lipid A in the OM

Less net negative charge (COL-R)

Periplasmic domain

Catalytic domain



5 membrane α-helices

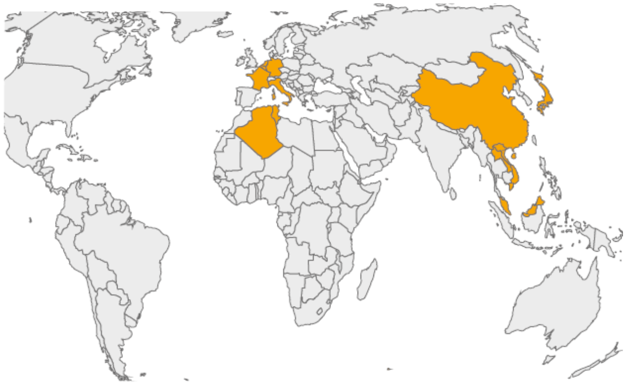
Zinc metalloprotein

Phenotypic tests (e.g., MIC with EDTA)

Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds

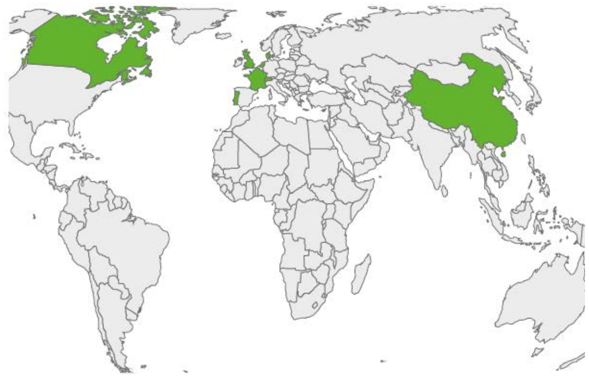
Skov and Monnet - Eurosurv. March, 2016

Food animals



Source	Year	Country	Type of specimen/ animal / infection	Isolates n (%)	Species
Food animals	1980s–2014	China	Chickens	104	*E. coli*
	2005–2014	France	Veal calves	106	*E. coli*
	2008–10	Japan	Pigs	2	*E. coli*
	2010–2011	Germany	Pigs	3	*E. coli*
	2010–2015	The Netherlands	Chickens, veal calves, turkeys	4 (< 1%)	*E. coli*
	2011	France	Pigs	1 (< 1%)	*E. coli*
	2011–12	Belgium	Pigs	6	*E. coli*
	2011–12	Belgium	Veal calves	7	*E. coli*
	2012	Laos	Pigs	3	*E. coli*
	2012	China	Pigs	31 (14%)	*E. coli*
	2012–13	Japan	Cattle	4	*E. coli*
	2013	Japan	Pigs	1	*Salmonella* Typhimurium
	2013	China	Pigs	68 (25%)	*E. coli*
	2013	Malaysia	Chickens	3	*E. coli*
	2013	Malaysia	Pigs	1	*E. coli*
	2013	France	Pigs	1 (< 1%)	*E. coli*
	2013	France	Chickens	3 (2%)	*E. coli*
	2013	France	Chickens (farm)	1	*Salmonella* 1,4 [5],12:i:-
	2014	France	Broilers	4 (2%)	*E. coli*
	2014	France	Turkeys	14 (6%)	*E. coli*
	2014	Italy	Turkeys	1	*E. coli*
	2014	China	Pigs	67 (21%)	*E. coli*
	2014	China	Chickens	1	*E. coli*
	2014–15	Vietnam	Pigs	9 (38%)	*E. coli*
	2015	Tunisia	Chickens	37 (67%)	*E.coli*
	2015	Algeria	Chickens	1	*E. coli*
Environment	2012	Switzerland	River water	1	*E. coli*
	2013	Malaysia	Water	1	*E. coli*

Food



Source	Year	Country	Type of specimen/ animal / infection	Isolates n (%)	Species
Food	2009	The Netherlands	Chicken meat	1	*E. coli*
	2009-2016	The Netherlands	Retail meat (mostly chicken and turkey)	47 (2%)	*E. coli*
	2010	Canada	Ground beef	2	*E. coli*
	2011	Portugal	Food product	1	*Salmonella* Typhimurium
	2011	China	Chicken meat	10 (5%)	*E. coli*
	2011	China	Pork meat	3 (6%)	*E. coli*
	2012–2014	Denmark	Chicken meat	5	*E. coli*
	2012	France	Chicken meat, guinea fowl pie	2	*Salmonella* Paratyphi B
	2013	France	Pork sausage	1	*Salmonella* Derby
	2013	China	Chicken meat	4 (25%)	*E. coli*
	2013	China	Pork meat	11 (23%)	*E. coli*
	2014	China	Chicken meat	21 (28%)	*E. coli*
	2014	China	Pork meat	29 (22%)	*E. coli*
	2014	The Netherlands	Chicken meat	2	*E. coli*
	2014	Switzerland	Vegetables	2	*E. coli*
	2012–2015	United Kingdom	Poultry meat	2	*Salmonella* Paratyphi B var Java

Most strains were 3GC-R and stored!

Humans

Source	Year	Country	Type of specimen/ animal / infection	Isolates n (%)	Species
Humans	2008	Vietnam	Dysentery	1	*Shigella sonnei*
	Before 2010	China	Faecal carriage	27 (7%)	NA
	2011	Canada	Gastrostomy tube	1	*E. coli*
	2011	The Netherlands	Bloodstream infection	1 (0.08%)	*E. coli*
	2012–2013	The Netherlands	Faecal carriage	6	*E. coli*
	NA	Sweden	Faecal carriage	2	*E. coli*
	2012	Thailand	Faecal carriage	2	*E. coli*
	2012	Laos	Faecal carriage	6	*E. coli*
	2012	Cambodia	Faecal carriage	1	*E. coli*
	2012–2015	United Kingdom	Salmonellosis	8	*Salmonella* Typhimurium
	2012–2015	United Kingdom	Salmonellosis	1	*Salmonella* Paratyphi B var Java
	2012–2015	United Kingdom	Salmonellosis	1	*Salmonella* Virchow
	2012–2015	United Kingdom	NA	3	*E. coli*
	2014	Germany	Wound infection (foot)	1	*E. coli*
	2014	China	Inpatient	13 (1%)	*E. coli*
	2014–2015	China	Bloodstream infection	2	*E. coli*
	2015	Denmark	Bloodstream infection	1	*E. coli*
	2015	Switzerland	Urinary tract infection	1	*E. coli*
	2015	China	Inpatient	3 (< 1%)	*K. pneumoniae*
	2015	China	Surgical site infection, peritoneal fluid	2	*K. pneumoniae*
	2015	China	Faecal carriage (children)	5 (2%)	*E. coli*

Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016

Eurosurveillance July, 2016

BB Xavier [1,2,3], C Lammens [1,2,3], R Ruhal [1,2,3], S Kumar-Singh [1,3,4], P Butaye [5,6,7], H Goossens [1,2,3], S Malhotra-Kumar [1,2,3]

53 colistin-R *E. coli* of porcine origin

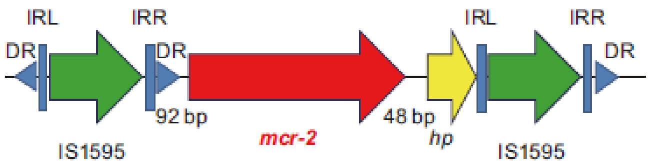
- *mcr-1* in 7 (13%)
- *mcr-2* in 11 (21%)

52 colistin-R *E. coli* of 52 bovine origin

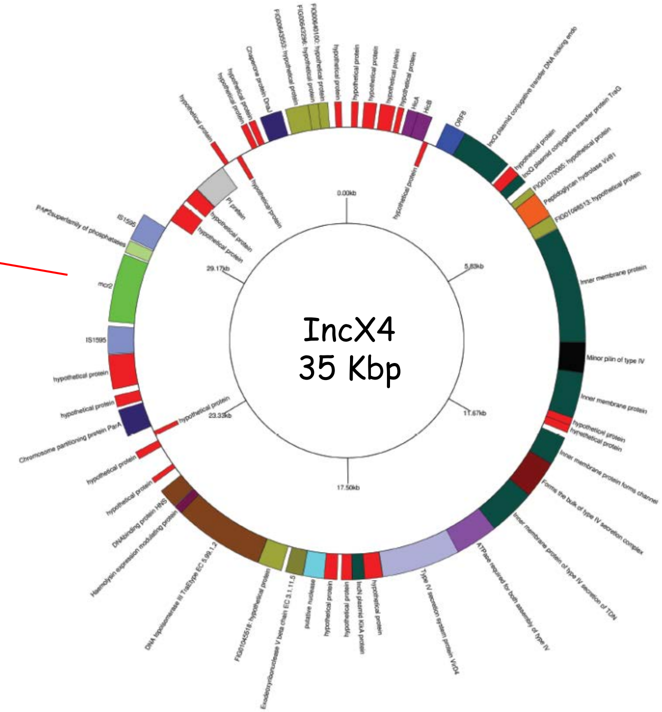
- *mcr-2* in 1 (2%)

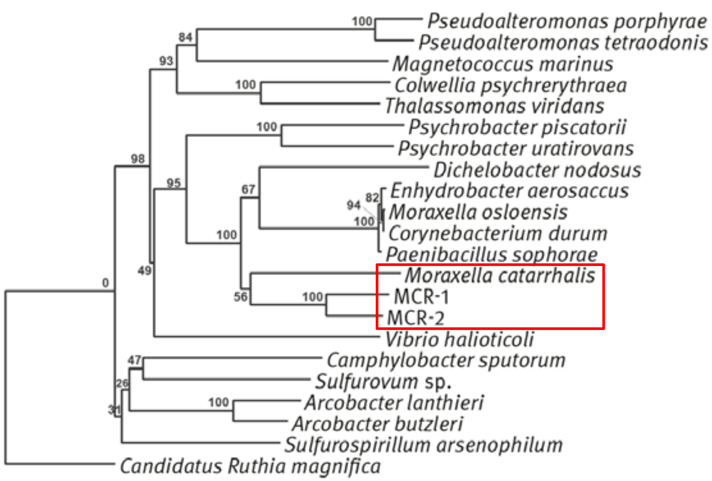
mcr-1 (1'626 bp) – *mcr-2* (1'617 bp): 77% nt identity

MCR-1 and MCR-2 proteins show 81% identity



Moraxella spp.





Phylogenetic analysis of the entire MCR-2 protein sequence

Identical to one found in *Salmonella enterica* (but *mcr*-negative)

Very high conjugation frequency (1.7×10^{-3})

Novel Plasmid-Mediated Colistin Resistance Gene *mcr-3* in *Escherichia coli*

mBio May/June 2017 Volume 8 Issue 3 e00543-17

Wenjuan Yin,[a] Hui Li,[a] Yingbo Shen,[a] Zhihai Liu,[a] Shaolin Wang,[a] Zhangqi Shen,[a] Rong Zhang,[b] Timothy R. Walsh,[c] Jianzhong Shen,[a] Yang Wang[a]

China, 2015

380 colistin-R *E. coli* from pig feces

- *mcr-3* in 7 (1.8%)

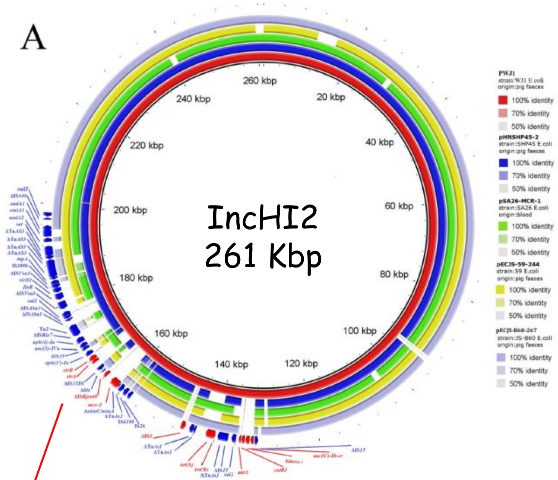
200 colistin-R *E. coli* from chicken cloacae

- No *mcr-3*

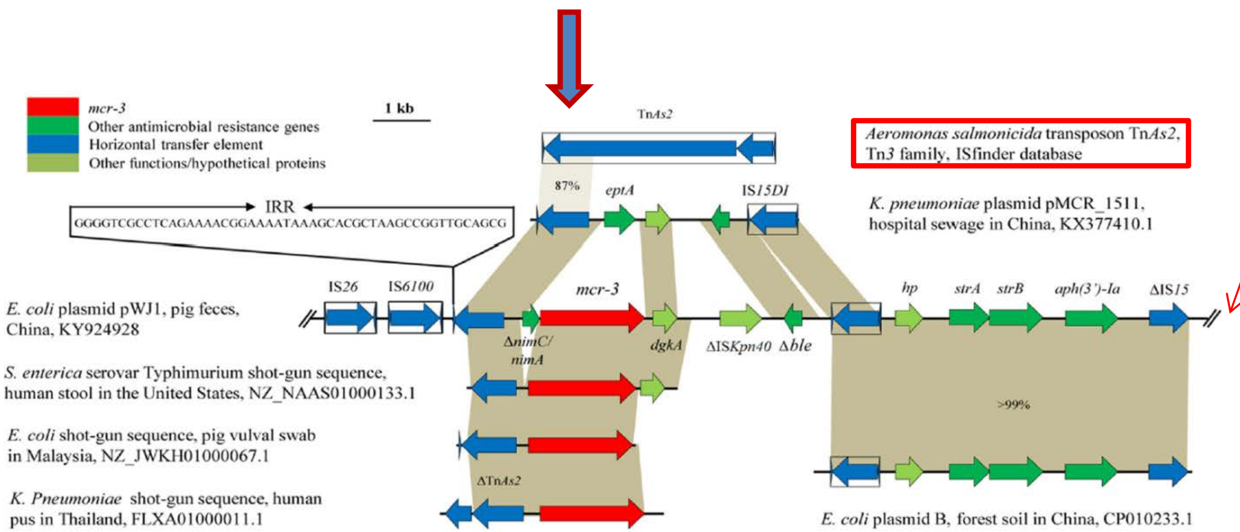
45% and 47% nt identity with *mcr-1* and *mcr-2*, respectively

33% and 32% aa identity with MCR-1 and MCR-2, respectively

MCR-3 showed 94-95% aa identity to proteins found in *Aeromonas* spp.



Very similar backbone compared to IncHI2 plasmids carrying *mcr-1*



Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016

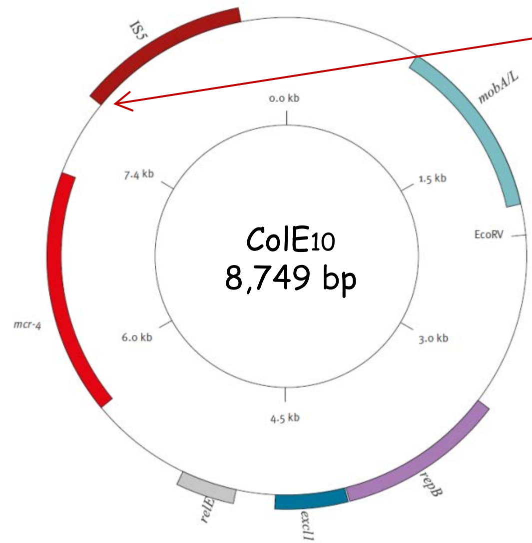
Eurosurv. Aug, 2017

A Carattoli [1], L Villa [1], C Feudi [1,2], L Curcio [3], S Orsini [3], A Luppi [4], G Pezzotti [3], CF Magistrali [3]

125 *E. coli* from piglets with diarrhea

- 50 were colistin-R
- 32 with *mcr-1*; 3 with *mcr-2*; 11 with *mcr-4*

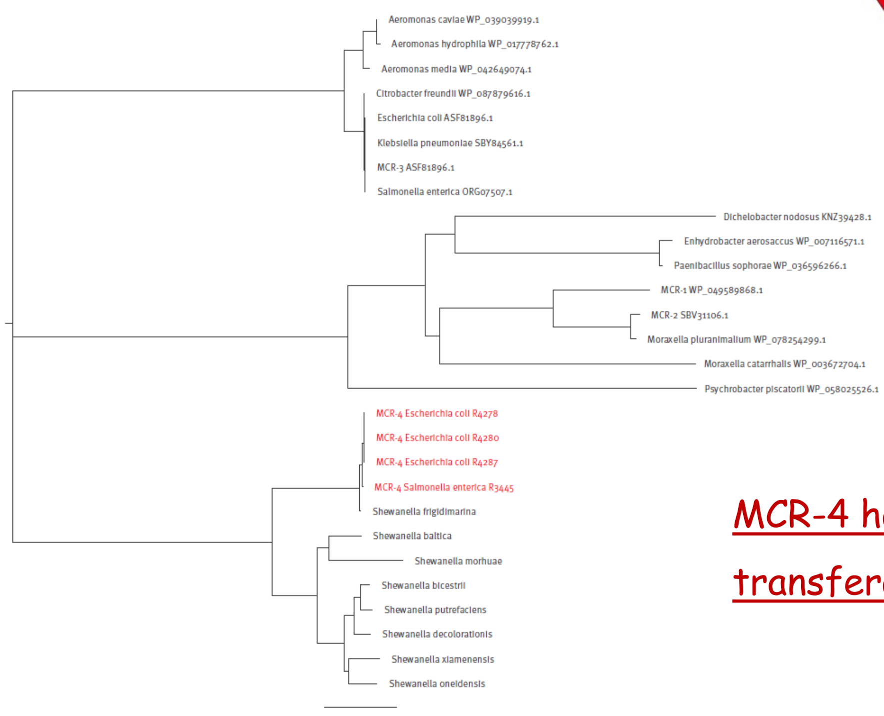
Strain	Country	MLST	Plasmid content
R3445 *Salmonella enterica*	Italy	34	ColE10, ColRNAI_34, ColRNAI_36, ColRNAI_38, ColRNAI_46
3445T (transformant in DH5-α)			ColE10
DH5-α			
R4287 *Escherichia coli*	Spain	10 (CC10)	I1, I2, FII, FIB, FIC, HI2, Col156, ColRNAI_34, ColE10
4287C (conjugant in CSH26 Rif[R])			ColE10, I2
CSH26 Rif[R]			
R4280 *E. coli*	Belgium	10 (CC10)	I1, FII, FIB, FIC, HI2, Col(MG828), ColRNAI_34, ColE10
R4278 *E. coli*	Belgium	7029	I1, X1, FII(pCoo), ColE10



IS5 flanked region: 99% identity with *Shew. frigidimarina*

Conjugation only in presence of IncI2 plasmid (helper)

Strain R4278
Plasmid integrated into chromosome; flanked by IS5



34%, 35% and 49% aa identity with MCR-1, MCR-2, and MCR-3, respectively

MCR-4 has 82-99% aa identity with phosphoethanolamine transferases found in *Shewanella* spp.

Early emergence of *mcr-1* in *Escherichia coli* from food-producing animals

*Zhangqi Shen, Yang Wang, Yingbo Shen, Jianzhong Shen, *Congming Wu*

www.thelancet.com/infection Vol 16 March 2016

I don't need to believe... because they are already here...

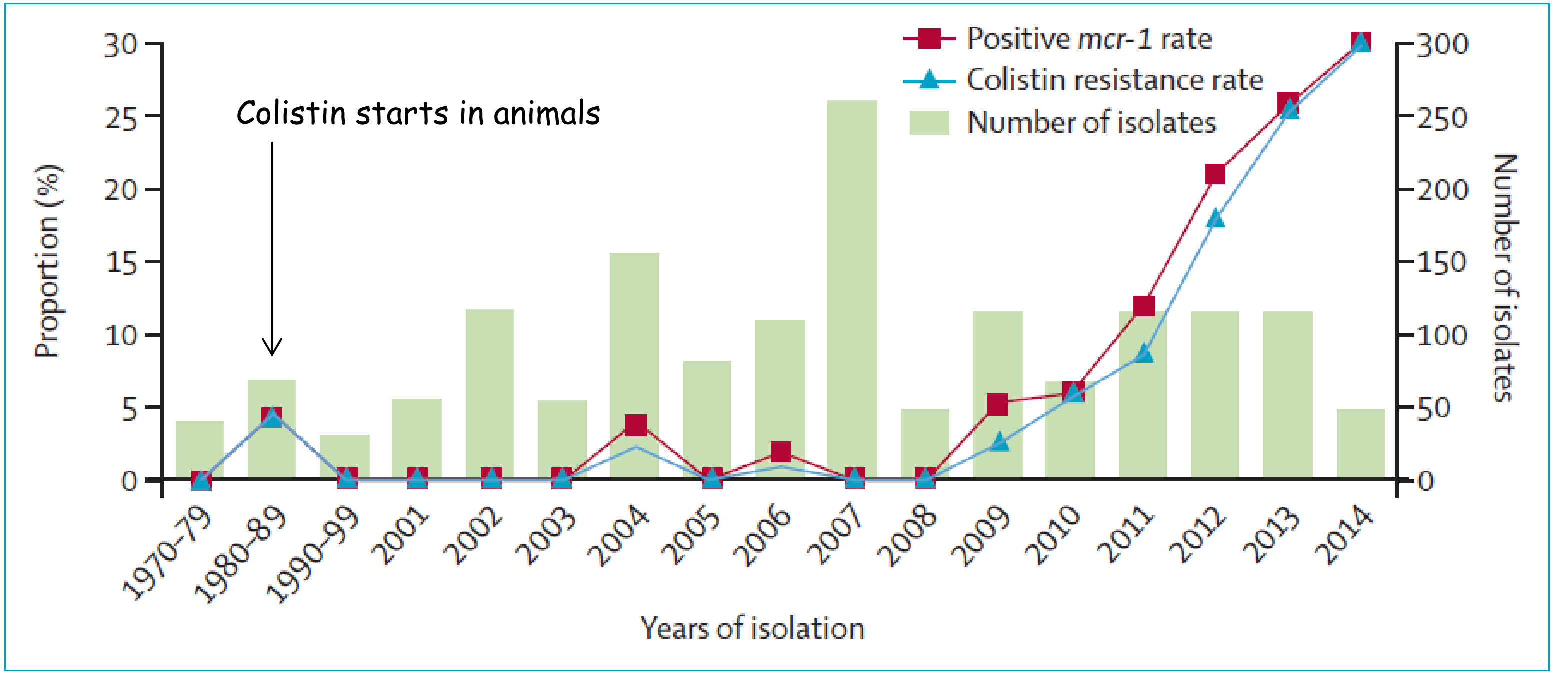


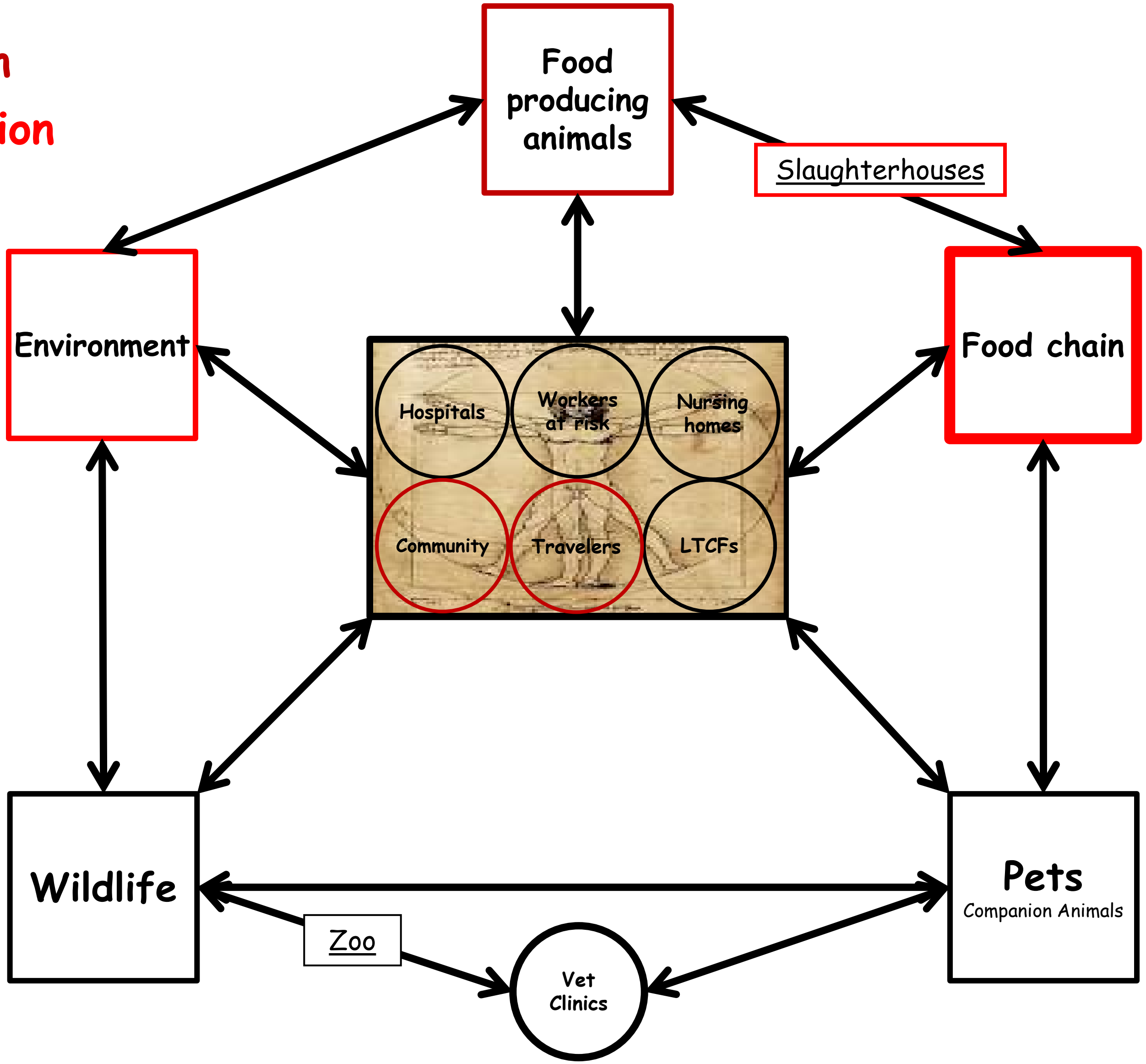
Figure: Presence of *mcr-1* and colistin resistance in *Escherichia coli* of chicken origin during 1970–2014

One Health Concept: *mcr* genes

Infection

Colonization

Contamination



High prevalence of the *mcr-1* gene in retail chicken meat in the Netherlands in 2015

Antimicrobial Resistance and Infection Control (2017) 6:83

Eefje J. A. Schrauwen[1,2*], Pepijn Huizinga[1,3], Nick van Spreuwel[1,2], Carlo Verhulst[1], Marjolein F. Q. Kluytmans-van den Bergh[1,4,5] and Jan A. J. W. Kluytmans[1,5,6]

Table 1 Determinants of the presence of *mcr-1* in Dutch retail chicken meat samples, 2015

Determinant	Samples n = 214	mcr-1 PCR positive n (%)	OR (95% CI)	Adjusted OR (95%CI)
Labelling as free-range				
Yes	70	10 (14.3)	reference	reference
No	144	43 (29.8)	2.6 (1.2-5.5)	3.0 (1.3-6.6)
Supermarket chain				
A	53	1 (1.9)	reference	reference
B	53	10 (18.9)	12.1 (1.5-98.3)	12.5 (1.5-101.8)
C	54	21 (38.9)	33.1 (4.2-257.8)	34.6 (4.4-272.0)
D	54	21 (38.9)	33.1 (4.2-257.8)	37.5 (4.8-295.3)
Country of origin[a]				
NL	67	21 (30.3)	2.9 (1.3-6.5)	
GER	44	19 (43.2)	4.8 (2.0-11.4)	
DEN	9	1 (11.1)	0.8 (0.1-6.9)	
NL/GER	80	11 (13.8)	reference	
NL/GER/BE	12	0 (0.0)	not applicable	
Unknown	2	1 (50.0)	6.3 (0.4-107.8)	

[a]*NL* The Netherlands, *GER* Germany, *DEN* Denmark, *BE* Belgium

Real-time PCR: 24.8%

Culture-based: 15.9%

only *mcr-1*

32 *E. coli*, 2 *K. pneumoniae*

We need further details on the production process (chain of production)

Spread of *mcr-1*-carrying Enterobacteriaceae in sewage water from Spain

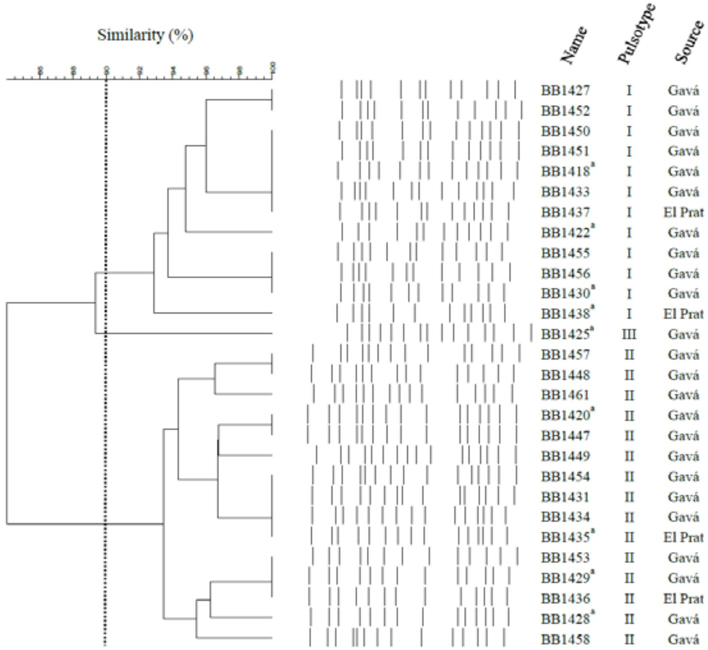
J Antimicrob Chemother 2017; **72**: 1050–1053

C. M. Ovejero[1]†, J. F. Delgado-Blas[1]†, W. Calero-Caceres[2], M. Muniesa[2] and B. Gonzalez-Zorn[1]*

Enterobacteriaceae from river (n=105) and sewage (n=90) around Barcelona (2013)

30 out of 90 from sewage were *mcr-1*-positive

Figure S1. Dendrogram of the 29 *E. coli* positive to *mcr-1*.



All with ESBL CTX-M-55

28/29 (pulsotypes I, II, III) were **ST1196**

Reported in carbapenemase producers from humans/dogs

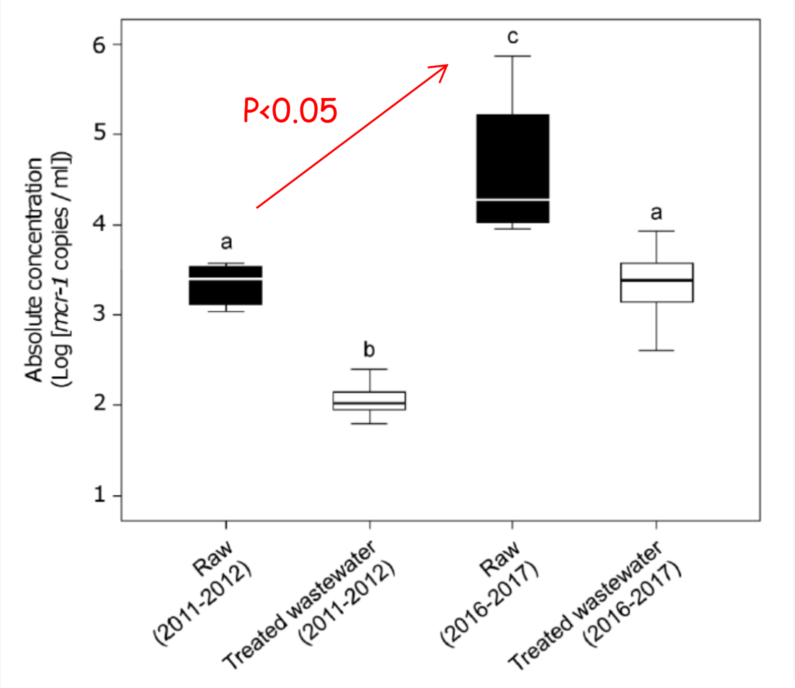
Title: Detection and quantification of the plasmid-mediated *mcr-1* gene conferring colistin resistance in wastewater

International Journal of Antimicrobial Agents 15-8-2017

Author: Itziar Lekunberri, José Luis Balcázar, Carles M. Borrego

SYBRGreen-based real-time PCR

Winter 2011 (Nov-Jan) *vs.* winter 2016 (Nov-Jan)



Evidence for the growing spread of *mcr-1*

Human infections/colonization with mcr-1-like producers

Detection of *mcr-1* among *Escherichia coli* Clinical Isolates Collected Worldwide as Part of the SENTRY Antimicrobial Surveillance Program in 2014 and 2015

September 2016 Volume 60 Number 9 Antimicrobial Agents and Chemotherapy

Mariana Castanheira, Michelle A. Griffin, Lalitagauri M. Deshpande, Rodrigo E. Mendes, Ronald N. Jones, Robert K. Flamm

Organism(s) and location where *mcr-1*-positive isolate(s) was observed	No. of *mcr-1*-positive isolates/ no. of colistin-resistant isolates (%)
All colistin-resistant organisms	19/390 (4.9)
E. coli	19/59 (32.2)
Belgium (Antwerp)	1/1
Brazil (Florianopolis)	1/1
Germany (Bonn and Kiel)	5/10
Hong Kong	1/1
Italy (Florence, Milan, and Rome)	4/9
Malaysia (Kelantan)	1/1
Poland (Warsaw)	1/2
Russia (Yekaterinburg)	1/2
Spain (Madrid)	3/3
USA (New York City)	1/15

[a] All *K. pneumoniae* isolates were negative for *mcr-1*.

E. coli: 13'526 strains

- Colistin-R: n=59, 0.4%
  - of which *mcr-1*: n=19, 0.1%

K. pneumoniae: 7'480 strains

- Colistin-R: n=331, 4.4%
  - of which *mcr-1*: n=0, 0%

Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract *Enterobacteriaceae* in Switzerland

Nadia Liassine [a], Laetititia Assouvie [b,c], Marie-Christine Descombes [d], Valérie Dénervaud Tendon [b,c], Nicolas Kieffer [b,c], Laurent Poirel [b,c], Patrice Nordmann [b,c,e,*]

International Journal of Infectious Diseases 51 (2016) 4–5

Urine sample. Feb-March, 2016:

2049 *Enterobacteriaceae* of which 1704 *E. coli*

Only one *E. coli mcr-1*: prevalence 0.05%

Screening for fecal carriage of MCR-producing *Enterobacteriaceae* in healthy humans and primary care patients

Zurfluh *et al. Antimicrobial Resistance and Infection Control* (2017) 6:28

1144 stool samples. July-Oct, 2016:

Colistin-R *Enterobacteriaceae*: prevalence 1.5-3.8%

No *mcr-1/-2* producers: prevalence 0.0%

Low prevalence of MCR-1-producing *Klebsiella pneumoniae* in bloodstream infections in China

Clinical Microbiology and Infection 6 August 2017

Beiwen Zheng, Hao Xu, Xiao Yu, Xiawei Jiang, Jing Zhang, Yunbo Chen, Jinwei Huang, Chen Huang, Yonghong Xiao

979 *K. pneumoniae* from 43 hospitals (2016):

Only two *mcr-1*-positive: prevalence 0.2%

MCR-1 and OXA-48 *In Vivo* Acquisition in KPC-Producing *Escherichia coli* after Colistin Treatment

August 2017 Volume 61 Issue 8 e02540-16 Antimicrobial Agents and Chemotherapy

Racha Beyrouthy,[a,b,c] Frederic Robin,[a,b,c] Aude Lessene,[d] Igor Lacombat,[e] Laurent Dortet,[f,g,h] Thierry Naas,[f,g,h] Valérie Ponties,[i] Richard Bonnet[a,b,c]

44-y man hospitalized in Portugal

VAP due to *A. baumannii*, *P. aeruginosa*, CP *K. pneumoniae*

Successfully treated with colistin for 20 days

After 2 months, ICU in France where

E. coli WI1 and WI2 were found in stools

	Strain	Carbapenemase-encoding genes	MIC (μg/ml) of[a]: AMX	FOX	CAZ	CTX	FEP	IPM	ETP	MEM	COL
ST1288 (identical)	WI1	bla_{KPC-3}	>256	16	>256	>32	12	4	6	2	0.25
	WI2	bla_{KPC-28}, bla_{OXA-48}	>256	8	>256	8	6	1	3	0.38	4 (*mcr*-1)
	E. coli DH5α-KPC-3	bla_{KPC-3}	>256	8	4	1	1	1	0.125	0.25	
	E. coli DH5α KPC-28	bla_{KPC-28}	64	8	64	0,5	1	0.125	0.032	0.032	
	E. coli DH5α		1	4	0.06	0.06	0.032	0.05	0.006	0.006	

E. coli WI2: contained two additional plasmids

- **IncX4, 33Kb: *mcr-1***
- IncL, 62Kb: bla_{OXA-48}

Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study

Wang Y et al., Lancet Infect Dis, Apr 2017

Infections

	mcr-1-negative *E coli* (n=508)	*mcr-1*-positive *E coli* (n=76)	Unadjusted p value*	Adjusted p value†	Adjusted p value‡
Region			0·006	0·208	0·044
Guangdong	348 (90%)	40 (10%)	..	..	..
Zhejiang	160 (82%)	36 (18%)	..	..	..
Age (years)	54·7 (18-17)	56·2 (17-85)	0·485		
Sex (male)	209 (41%)	47 (63%)	0·001	0·010	0·011
Site of infection	..	..	0·074	0·513	0·513
Respiratory	54 (11%)	16 (21%)	..		
Bloodstream	73 (14%)	8 (11%)	..	..	..
Skin and soft tissue	37 (7%)	8 (11%)	..	..	..
Intra-abdominal	141 (28%)	22 (29%)	..	..	..
Urinary tract	199 (39%)	22 (29%)	..	..	..
Bone or joint	0	0	..	..	..
CNS	4 (1%)	0	..	..	..
Admitted to ward (intensive care unit)	38 (8%)	8 (11%)	0·358	..	..
Comorbidities and risk factors					
Immunosuppression	30 (6%)	11 (15%)	0·006	0·011	0·011
Diabetes	80 (16%)	9 (12%)	0·377	..	..
Neutropenia	11 (2%)	1 (1%)	1·000	..	..
Artificial lung ventilation	18 (4%)	2 (3%)	1·000	..	..
Vascular catheter	131 (26%)	22 (29%)	0·559	..	..
Abdominal or pelvic catheter	40 (8%)	2 (3%)	0·099	0·096	0·114
Urinary catheter	128 (25%)	24 (32%)	0·237	..	..
Antibiotic use in the past 3 months	405 (80%)	74 (97%)	0·0009	0·008	..
Type of antibiotic used in the past 3 months					
Penicillin and β-lactamase inhibitor combination	102 (20%)	23 (30%)	0·043	..	0·559
Cephalosporin (narrow)	20 (4%)	4 (5%)	0·538	..	..
Cephalosporin (broad)	127 (25%)	23 (30%)	0·327	..	..
Carbapenem	45 (9%)	18 (24%)	0·0001	..	0·002
Aminoglycosides	21 (4%)	3 (4%)	1·000	..	..
Fluoroquinolones	95 (19%)	23 (30%)	0·019	..	0·017

Table 2: Risk factors and associated outcomes of *mcr-1*-positive *Escherichia coli* infection

Colonization

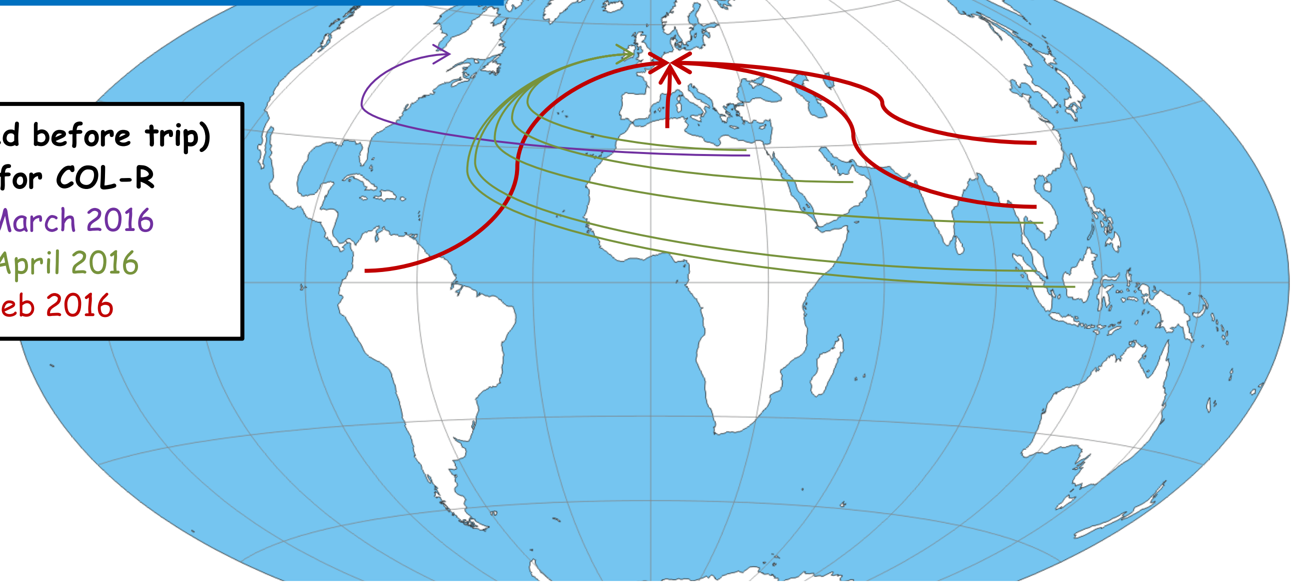
	mcr-1-negative *E coli* (n=378)	*mcr*-1-positive *E coli* (n=35)	Unadjusted p value*	Adjusted p value†
Region			0·19	..
Guangdong	128 (34%)	8 (23%)	..	..
Zhejiang	250 (66%)	27 (77%)	..	..
Age (years)	60 (10–82)	55 (27–89)	0·92	..
Sex				
Male	197 (52%)	21 (60%)	0·37	..
Female	181 (48%)	14 (40%)	..	..
Admitting ward				
Non-intensive care unit	332 (88%)	31 (89%)	0·89	..
Intensive care unit	46 (12%)	4 (11%)	..	..
Living place				
City/county	216 (57%)	17 (49%)	0·33	..
Village	162 (43%)	18 (51%)	..	..
Proximity to commercial animal farm				
<1 km	142 (38%)	10 (29%)	0·03	0·995
1–10 km	62 (16%)	12 (34%)	..	..
>10 km	174 (46%)	13 (37%)	..	..
Education				
None/primary	166 (43·9%)	22 (63%)	0·09	0·052
Secondary	147 (38·9%)	9 (26%)	..	..
Tertiary	65 (17·2%)	4 (11%)	..	..
Income class				
Low	166 (44%)	20 (57%)	0·13	..
Middle/high	212 (56%)	15 (43%)	..	..
Diets				
Vegetarian	11 (3%)	0 (0%)	0·61	..
Non-vegetarian	367 (97%)	35 (100%)	..	..
Antibiotic use before hospital stay				
Yes	88 (23%)	20 (57%)	0·0008	<0·0001
No	243 (64%)	15 (43%)	..	..
Not sure	47 (12%)	0	..	..
Antibiotic use during hospital stay‡				
Yes	318 (84%)	32 (91%)	0·25	..
No	60 (16%)	3 (9%)	..	..
Drinking water				
Municipal	316 (84%)	30 (86%)	0·75	..
Non-municipal (vendor, well, or other)	62 (16%)	5 (14%)	..	..

Table 3: Risk factors associated with *mcr-1*-positive *Escherichia coli* colonisation in patients in hospital

Travelers Can Import Colistin-Resistant *Enterobacteriaceae*, Including Those Possessing the Plasmid-Mediated *mcr-1* Gene

Antimicrobial Agents and Chemotherapy
August 2016 Volume 60 Number 8

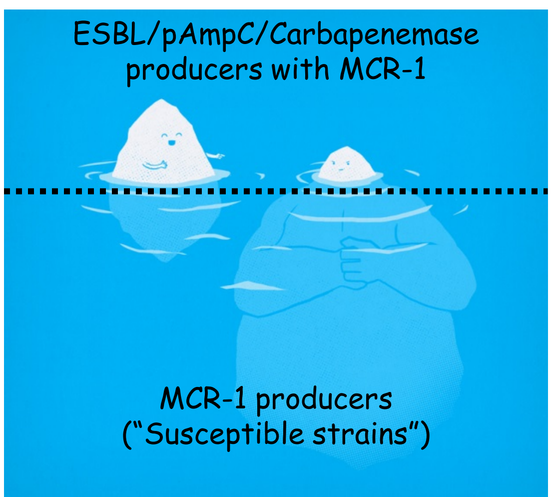
Odette J. Bernasconi,[a,b] Esther Kuenzli,[c,d] João Pires,[a,b] Regula Tinguely,[a] Alessandra Carattoli,[a,e] Christoph Hatz,[c,d] Vincent Perreten,[f] Andrea Endimiani[a]



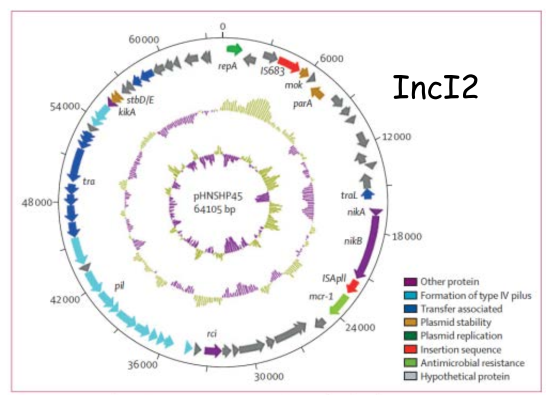
Sporadic cases (not screened before trip)
Only 3GC-R strains tested for COL-R

- Mulvey MR et al., LID, March 2016
- Doumith M et al., JAC, April 2016
- Arcilla MS *et al.*, LID, Feb 2016

What is the real prevalence of MCR-1 producers?



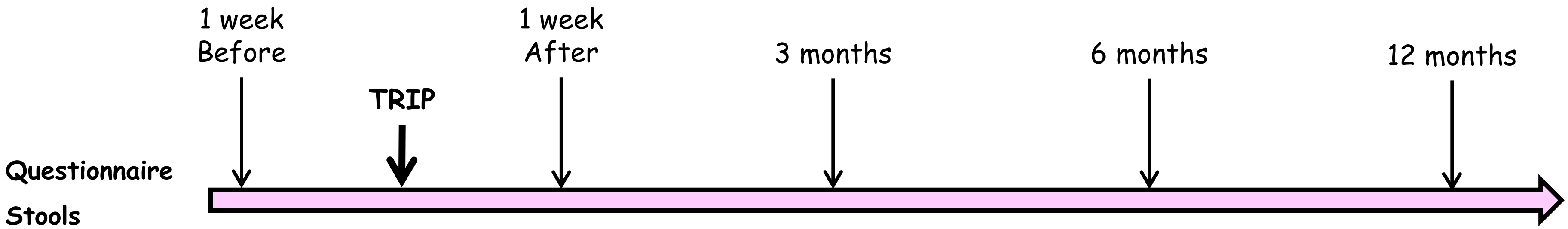
Plasmids carrying only *mcr-1* (non-ESC-R)

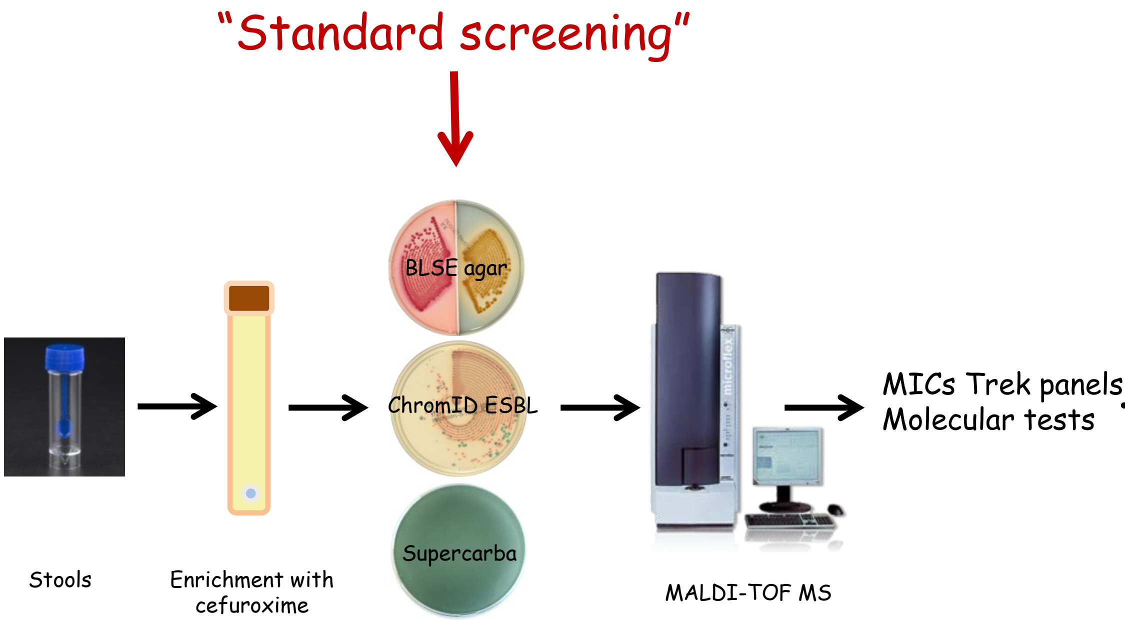


IncP
IncX4
IncI1
IncHI2

Liu YY et al., LID, 16:161, 2016

38 HVs living in Switzerland and traveling to India (Jan-Aug 2015)





Before trip:

- 3/38 (**~8%**) with CTX-Ms *E. coli*

After trip:

- 29/38 (**~76%**) with ESC-R-*Enterobacteriaceae*
  - 26/38 with CTX-Ms *E. coli*
  - 3/38 with DHA *E. coli*

At 3 months:

- 11/34 (**~32%**) with ESC-R-*Enterobacteriaceae*
  - 10/34 with CTX-Ms *E. coli*
  - 1/34 with CMY-2-like *E. coli*

At 6 months:

- 8/31 (**~26%**) with ESC-R-*Enterobacteriaceae*
  - 5/31 with CTX-Ms *E. coli*
  - 3/31 with cAmpC, CMY-2-like *E. coli*

At 12 months:

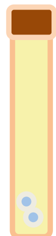
- 4/25 (**~16%**) with ESC-R-*Enterobacteriaceae*
  - All CTX-Ms

All ESBL/pAmpC strains were:

- **polymyxins-S (MICs ≤1 mg/L)**
- **PCR negative for *mcr-1***

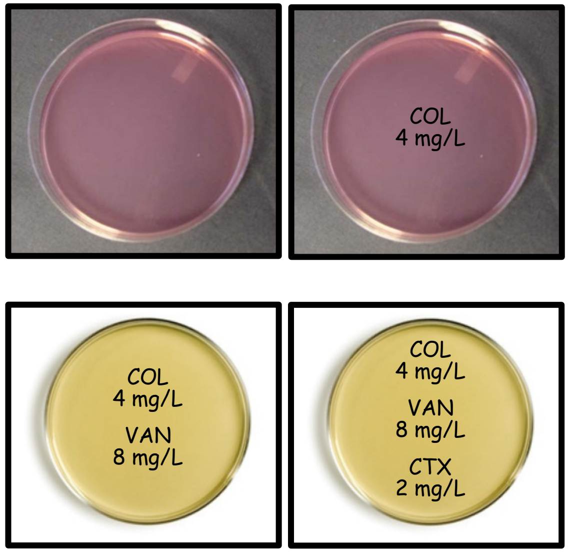
New screening strategy for COL-R strains

Stools →



Enrichment with **Colistin, COL (2 mg/L)** →

MacConkey



Problem: species naturally R to COL

ChromAgar Orientation

→

MALDI-TOF MS

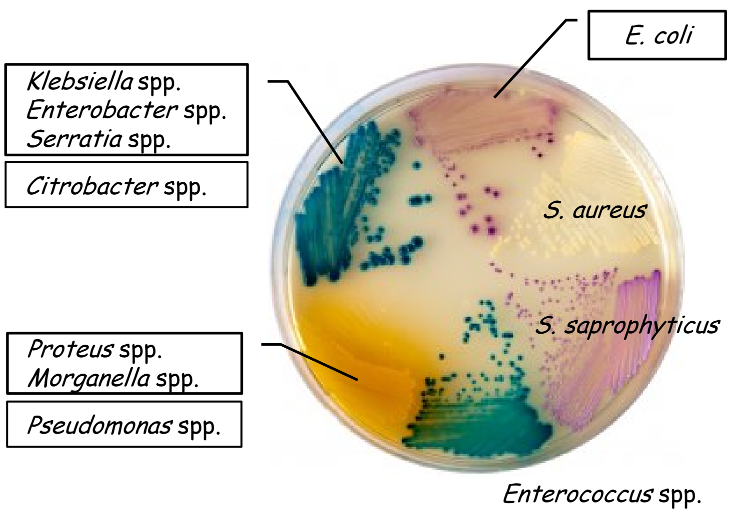
→

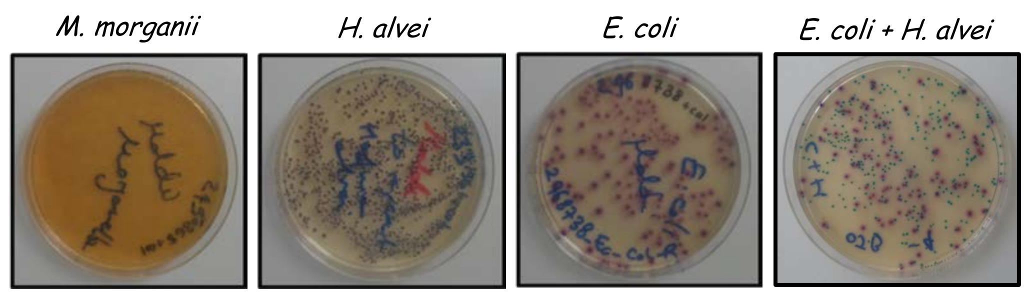
PCR for *mcr-1/2*

MICs Trek panels

Further molecular tests

- Phylogenetic-group
- MLST
- PBRT
- Conjugation
- Transformation
- WGS/WPS





New screening strategy for COL-R strains

Before | **TRIP** | After | 3 months | 6 months | 12 months

ID	Age/ Sex	Trips last year	Stools #1	Days in India	Stools #2	Stools #3	Stools #4	Stools #5
18	52 F	TUR ITA GER FRA POR	-	9	-	*E. coli* **non-ESC-R** B2-ST141 COL MIC = 6 mg/L PCR *mcr-1* = neg	-	-
19	53 F	ITA	-	20	-	-	-	* *E. coli* **non-ESC-R** A/B1-ST5 COL MIC = 6 mg/L **PCR *mcr-1* = POS**
26	50 F	ITA FRA	*E. coli* **non-ESC-R** D1-ST69 COL MIC = 4 mg/L PCR *mcr-1* = neg	14	-	-	-	-
56	42 M	ICE BEL UK	-	9	-	*E. coli* CTX-M New-ST COL MIC = 8 mg/L PCR *mcr-1* = neg	-	-
78	57 F	SPA ITA AUT	-	20	*E. coli* CTX-M D2-New-ST COL MIC = 12 mg/L PCR *mcr-1* = neg	Drop out	Drop out	Drop out
86	53 F	SPA POR ISR	-	15	*E. coli* DHA B1-ST3075 COL MIC = 6 mg/L PCR *mcr-1* = neg	-	-	Drop out
88	25 M	NED ITA POL	-	8	*E. coli* CTX-M D1-New-ST COL MIC = 4 mg/L PCR *mcr-1* = neg	-	-	-
100	57 F	-	-	14	*E. coli* **non-ESC-R** A1-ST10 COL MIC = 12 mg/L **PCR *mcr-1* = POS**	-	-	-

COL-R 1/38 (2.6%)
MCR-1 0/38

COL-R 4/38 (10.5%)
MCR-1 1/38 (2.6%)

Overall, no other species (only *E. coli*)

Heterogeneous Genetic Location of *mcr-1* in Colistin-Resistant *Escherichia coli* Isolates from Humans and Retail Chicken Meat in Switzerland: Emergence of *mcr-1*-Carrying IncK2 Plasmids

Antimicrobial Agents and Chemotherapy November 2017 Volume 61 Issue 11

Valentina Donà,[a*] Odette J. Bernasconi,[a,b] João Pires,[a,b] Alexandra Collaud,[c] Gudrun Overesch,[c] Alban Ramette,[a] Vincent Perreten,[c] Andrea Endimiani[a]

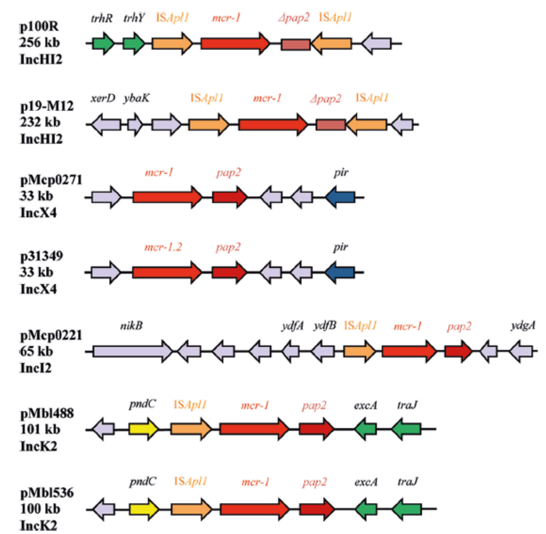
Prevalence of mcr-1 producers:

- HIV+, 1%
- Travelers, 5.2%
- Chicken meat from Germany, 1.1%
- Swiss chicken meat, 0%

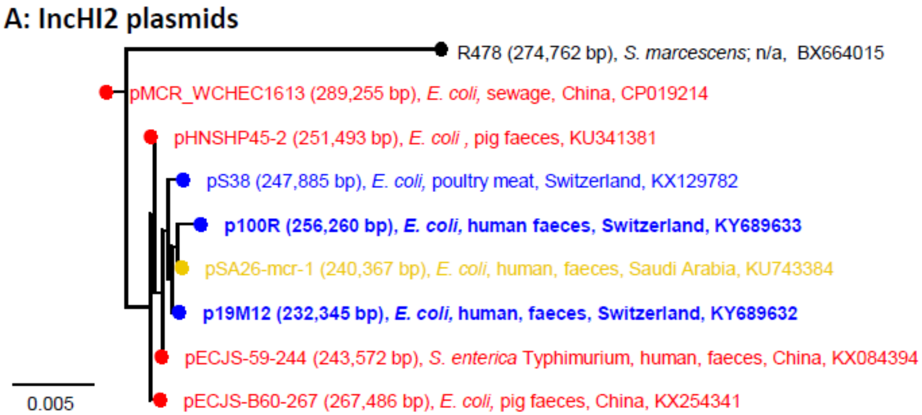
9 *mcr-1 E. coli* (3 humans; 6 meat)

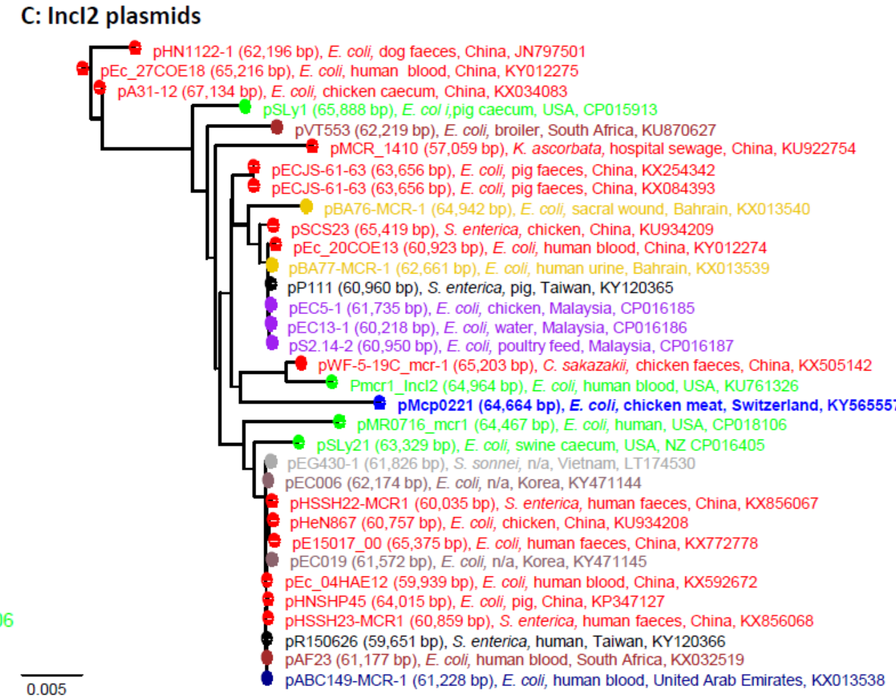
- **7 plasmid-mediated**
- **2 chromosomally located**

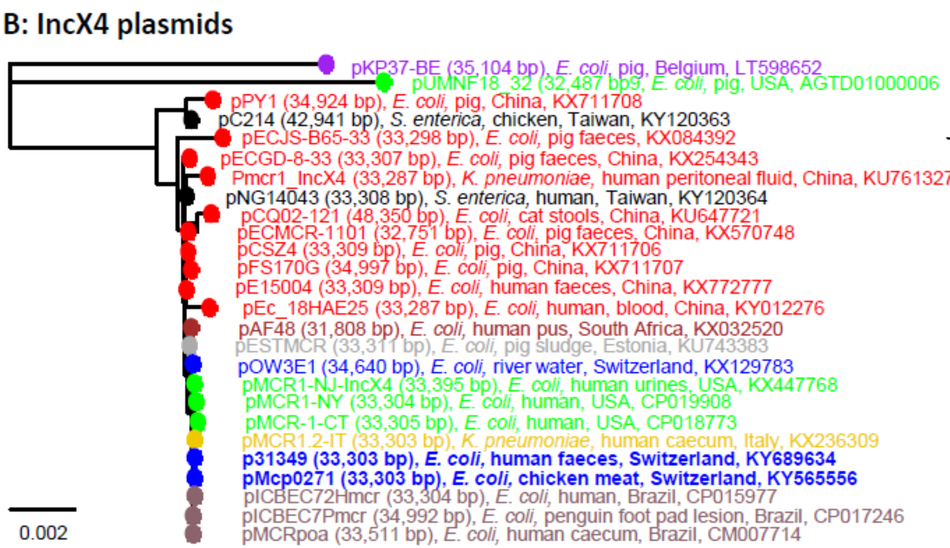
E. coli strain	Source[g]	ST[a]	Collection period	Antibiotic resistance[b]	Colistin MIC (μg/ml)[c]	Plasmid[d]	Plasmidic resistance gene(s)[e]	*mcr-1* location (plasmid length in kb)
100R	Human feces (traveler)	10	2015	CST, PMB, LVX, CIP, DOX, SXT	≥8	IncHI2	*aadA1, aadA2, bla*$_{\text{TEM-1}}$*, mcr-1, mph(A), sul3, tetA, dfrA15, dfrA14*	IncHI2 (256)
						IncFII/FIB	*bla*$_{\text{TEM-1}}$*, aadA2, aadA24, aph(3)-Ia, qnrS1, cmlA1, sul3, tetA, dfrA12*	
						IncX1		
						ColRNAI		
19-M12	Human feces (traveler)	5	2015	CST, PMB, DOX, SXT	≥8	IncHI2	*aadA2, bla*$_{\text{TEM-1}}$*, mcr-1, mph(A), cmlA1, sul3, tetA, dfrA12, dfrA14*	IncHI2 (232)
						IncFII/FIB	*bla*$_{\text{TEM-1}}$*, aadA1, qnrS1, tetA, dfrA1*	
31349	Human feces (HIV+ subject)	5	2015	CST, PMB	≥8	IncX4	*mcr-1.2*	IncX4 (33)
						IncL/M		
						IncFII		
						IncFIB		
Mcp0271	Retail chicken meat	58	2016	CST, AMP	4	IncX4	*mcr-1*	IncX4 (33)
						IncFII/FIB/FIA	*bla*$_{\text{TEM-1}}$	
Mcp0221	Retail chicken meat	1775	2016	CST	4	IncI2	*mcr-1*	IncI2 (65)
Mbl488	Retail chicken meat	38	2014	CST, SMX, TMP, NAL, CAZ, CTX, AMP, TET	8	IncK2	*bla*$_{\text{TEM-1}}$*, mcr-1, sul2*	IncK2 (101)
3GC-R						IncFII/FIB	*aadA1, bla*$_{\text{TEM-1}}$*, sul1, tetA, dfrA1*	
						IncI1	*aadA5, bla*$_{\text{CTX-M-1}}$*, sul2, dfrA17*	
						po111		
Mbl536	Retail chicken meat	226	2014	CST, SMX, TMP, CIP, TET, NAL, AMP	4	IncK2	*bla*$_{\text{TEM-1}}$*, mcr-1, sul2*	IncK2 (101)
						IncI1	*bla*$_{\text{TEM-1}}$*, sul2, tetA, dfrA1*	
						IncX1	*bla*$_{\text{TEM-52}}$	
Mbl323	Retail chicken meat	38	2014	CST, CAZ, AMP, NAL, CTX	8	IncK2	*bla*$_{\text{CMY-2}}$	Chromosome
3GC-R						IncN	*aadA22, lnuF*	
						IncFII/FIBColRNAI		
Mbl506[f]	Retail chicken meat	1049	2014	CST, TET, NAL, CTX, CHL, SMX, TMP, CAZ, AMP	8	IncFII/FIB	*tetA*	Chromosome[i]
3GC-R						IncI1	*aadA1, erm(42)*	
						IncI2	*aad24, bla*$_{\text{TEM-1}}$	
						IncQ1[h]	*aad24, strA, strB, mph(B), sul1, sul2, tetA, dfrA1*[h]	



Most with *ISApI1*



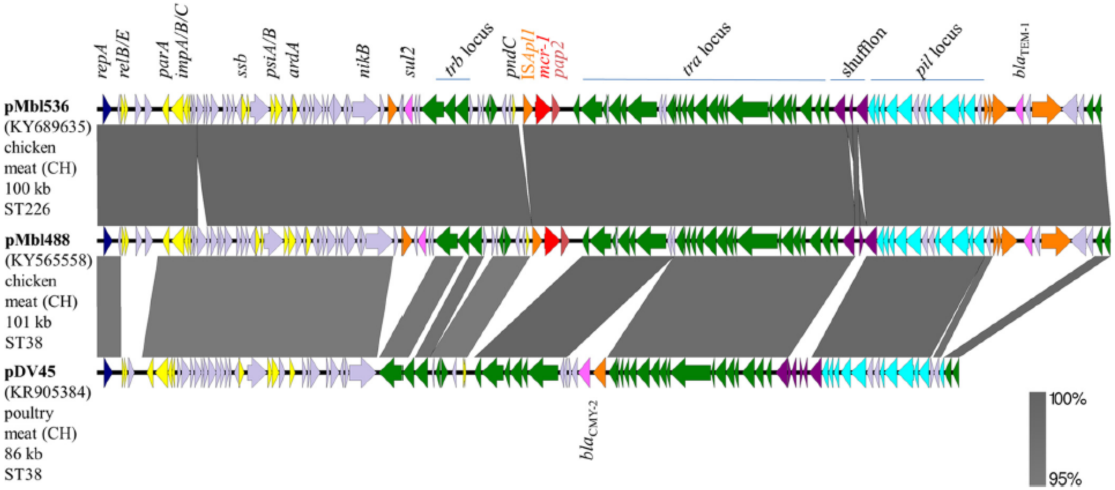




Very similar or identical plasmids (highly stable!)

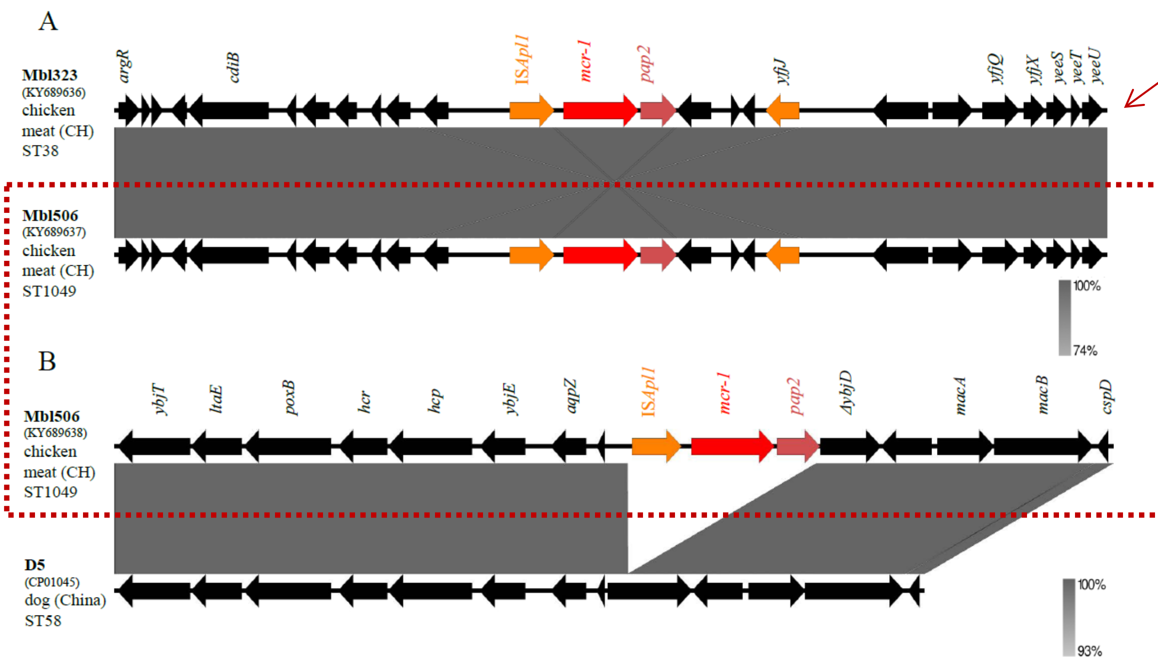
Most are conjugative

In blue from this study or Switzerland



CMY-2-IncK2 plasmids represents a major attack in EU chicken production

They are frequently carried by a **hyperepidemic ST38 *E. coli* clone** transmitted to humans



One chromosomal copy (in a ST38 *E. coli*)

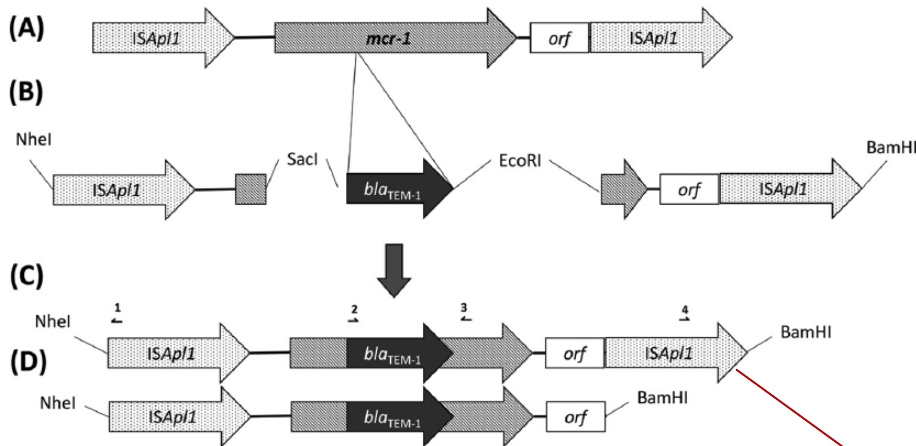
Two chromosomal copies

In Vitro Study of IS*Apl1*-Mediated Mobilization of the Colistin Resistance Gene *mcr-1*

Laurent Poirel,[a,b,c] Nicolas Kieffer,[a,b,c] Patrice Nordmann[a,b,c,d]

Antimicrobial Agents and Chemotherapy July 2017 Volume 61 Issue 7

IS*Apl1* first identified in
Actinobacillus pleuropneumoniae (*Pasteurellaceae*)
Porcine necrotic pleuropneumonia

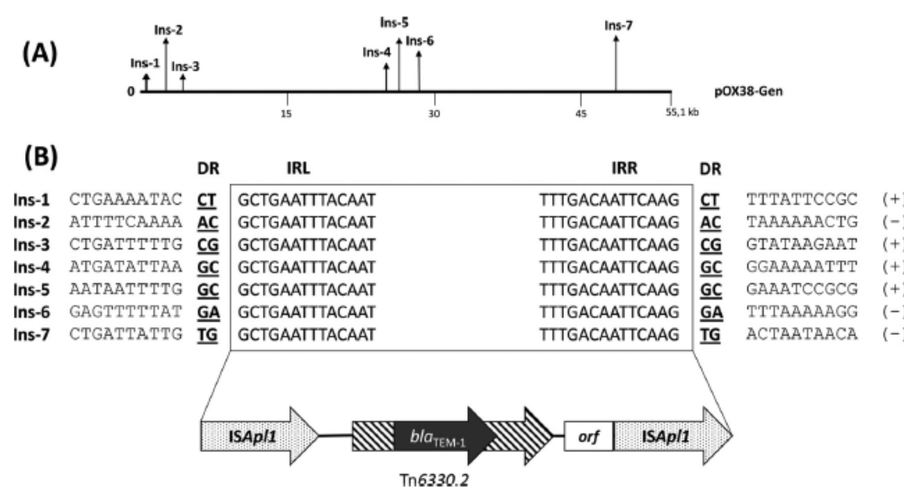


2,600 bp **composite transposon** (?)
[Tn*6330.2*]

Creation of two truncated forms

No transposition events

Transposition frequency (2.2 x 10^{-8})



Colistin and Polymyxin B Susceptibility Testing for Carbapenem-Resistant and *mcr*-Positive *Enterobacteriaceae*: Comparison of Sensititre, MicroScan, Vitek 2, and Etest with Broth Microdilution Chew KL, JCM, Sep 2017

CLSI, 2017: S ≤ 2 mg/L – R ≥ 2 mg/L (*Ec, Kp, Ea, Ecl, Ro*)

EUCAST, 2017: S ≤ 2 mg/L – R > 2 mg/L

Both recommend broth microdilution (BMD)

76 *Enterobacteriaceae* (21 *mcr-1*)

Sensitivities for detecting *mcr-1*-positive strains

Drug	Method	% of isolates susceptible at a breakpoint of ≤2 mg/liter	% of isolates susceptible at a breakpoint of ≤1 mg/liter
Colistin	BMD	71.4	90.5
	Sensititre	100	100.0
	Vitek 2	42.9	95.2
	Etest	76.2	95.2
	MicroScan	100	NA

Should we lower cutoff?

Performance in comparison to BMD

Drug	Method	No. of isolates that were susceptible	No. of isolates that were resistant	No. (%) of isolates exhibiting EA	No. (%) of isolates exhibiting CA	No. of isolates exhibiting VMEs (%)	No. of isolates exhibiting MEs (%)	Spearman's coefficient
Colistin	Vitek 2	60	16	71 (93.4)[b]	67 (88.2)	9 (36.0)	0 (0)	0.873[c]
	Sensititre	46	30	68 (89.5)	69 (90.1)[b]	1 (4)	6 (11.8)	0.863[c]
	Etest	51	25	57 (75.0)	70 (92.1)[b]	3 (12.0)	3 (5.9)	0.600[c]
	MicroScan	44	32	NA[d]	67 (88.2)	1 (4)	8 (15.8)	NA[d]

Performance of simulated breakpoints

Simulated breakpoints	Drug	Method	No. of isolates that were: Susceptible	Resistant	Intermediate	No. (%) of isolates exhibiting: CA	VMEs	MEs	mEs
Susceptible at ≤2 mg/liter; intermediate at 4 mg/liter; resistant at ≥8 mg/liter	Colistin	Vitek 2	60	12	4	62 (81.6)	2 (14.3)	0 (0.0)	11 (14.5)
		Sensititre	46	29	1	58 (76.3)	1 (7.1)	5 (9.8)	12 (15.8)
		Etest	51	9	16	63 (82.9)	1 (7.1)	1 (2.0)	9 (11.8)
		MicroScan	44	29	3	56 (73.7)	1 (7.1)	5 (9.8)	14 (18.4)
Susceptible at ≤1 mg/liter; intermediate at 2 mg/liter; resistant at ≥4 mg/liter	Colistin	Vitek 2	49	16	11	66 (86.8)	2 (8.0)	0 (0.0)	6 (7.9)
		Sensititre	46	30	0	69 (90.8)	1 (4.0)	2 (4.3%)	4 (5.3)
		Etest	46	25	5	69 (90.8)	1 (4.0)	1 (2.1)	5 (6.6)
		MicroScan	NA	NA	NA	NA	NA	NA	NA

A simple phenotypic method for screening of MCR-1-mediated colistin resistance

Clinical Microbiology and Infection 12 August 2017

Marco Coppi, Antonio Cannatelli, Alberto Antonelli, Ilaria Baccani, Vincenzo Di Pilato, Samanta Sennati, Tommaso Giani, Gian Maria Rossolini

Table 1. Bacterial strains tested in this study, and colistin MIC values measured by broth microdilution in absence or presence of 900 mg/mL DPA (Colistin-MAC test). For a detailed description of tested strains and results see Table S1.

Species	Mechanism of Colistin resistance[a]	No. of strains	MIC Colistin (µg/mL) (median value)	MIC Colistin (µg/mL) + DPA 900 µg/ml (median value)	Fold of MIC reduction
Escherichia coli	*mcr-1*/ *mcr-1*-like	53	4 - >8 (8)	≤0.125 - 1 (≤0.125)	8 - ≥128
	mcr-NEG, n.d.	9	4 - 8 (8)	4 - >8 (8)	≈[b]
Escherichia coli J53AZI^{R} Transconjugants	*mcr-1*-like	3	4 (4)	≤0.125 - 0.5 (0.25)	8 - ≥32
Klebsiella pneumoniae	*mcr-1*-like/*mcr-1.2*	2	8 - >8	>8	≈[b]
	mcr-NEG PmrB mutant/inactivated *mgrB*/n.d.	4	>8	>8	≈[b]
Citrobacter braakii	*mcr-1*	1	8	0.5	16
Citrobacter freundii complex	*mcr-1*	1	8	≤0.125	≥64
Enterobacter cloacae complex	*mcr-1*	1	>8	≤0.125	≥128

74 colistin-R *Enterobacteriaceae* (61 with *mcr-1*-like)

↓

Cation-adjusted MH broth (CLSI)

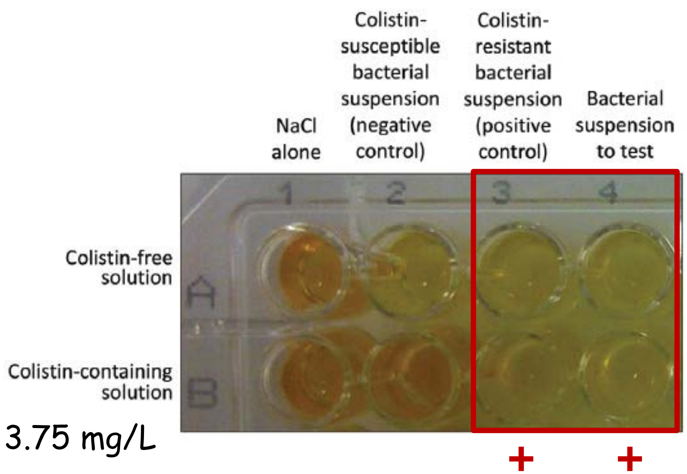
A MIC reduction of at least 8-fold dilution

Not for disk!

Rapid Detection of Polymyxin Resistance in *Enterobacteriaceae*

Patrice Nordmann, Aurélie Jayol, Laurent Poirel

Carbohydrate metabolism
Acid formation observed with pH indicator (phenol red)



3.75 mg/L

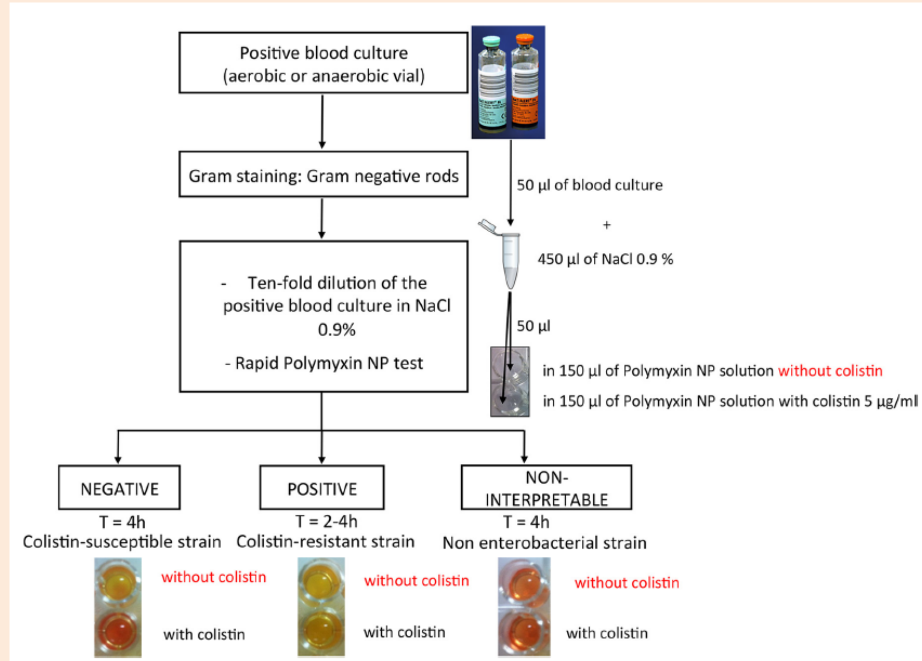
<2 hrs

RAPID POLYMYXIN NP

~10 Euro

Rapid Detection of Polymyxin-Resistant *Enterobacteriaceae* from Blood Cultures

Jayol A. *et al.*, J Clin Micro; 54:9, 2016



Final results in 2-4 hrs

Sens and Spec: 98% and 100%, respectively

Comparison of methods for detection of plasmid-mediated and chromosomally-encoded colistin resistance in Enterobacteriaceae

Jayol A. et al. CMI, June 2017

123 *Enterobacteriaceae*:
83 were colistin-R

BMD, gold standard

BD Phoenix automated system

- 10 Very Major Errors (12%)

Rapid Polymyxin NP test

- 1 Very Major Error (1.2%)
- 1 Major Error (1.2%)

Isolate	Species (number of isolates)	Phenotype	Mechanism of resistance to colistin[a]	BMD MIC colistin	Phoenix MIC colistin	Phoenix Discrepancies[b]	Rapid Polymyxin NP test Result	Rapid Polymyxin NP test Discrepancies[b,c]
Isolates susceptible to colistin								
ATCC25922	*E. coli*	S	NA	0.25	≤0.5	No	-	No
2 to 15	*E. coli* (n= 14)	S	NA	0.12 to 0.5	≤0.5	No	-	No
16 to 26	*K. pneumoniae* (n=11)	S	NA	0.12 to 2	≤0.5	No	-	**Yes, ME (n=1)**
27 to 29	*K. oxytoca* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
30 to 32	*E. cloacae* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
33	*E. asburiae*	S	NA	0.12	≤0.5	No	-	No
34	*E. aerogenes*	S	NA	0.12	≤0.5	No	-	No
35 to 37	*C. freundii* (n=3)	S	NA	0.25	≤0.5	No	-	No
38 to 40	*C. koseri* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
Isolates resistant to colistin								
41	*M. morganii*	R	Intrinsic	>128	>4	No	+	No
42-43	*P. mirabilis* (n=2)	R	Intrinsic	>128	>4	No	+	No
44	*P. vulgaris*	R	Intrinsic	>128	>4	No	+	No
45	*P. stuartii*	R	Intrinsic	>128	>4	No	+	No
46 to 48	*S. marcescens* (n=3)	R	Intrinsic	>128	>4	No	+	No
49 to 52	*H. alvei* (n=4)	R	Intrinsic	8 or 16	4 or >4	No	+	No
53 to 56	*H. paralvei* (n=4)	R	Intrinsic	8	4 or >4	No	+	No
57 to 68	*E. coli* (n= 11)	R	Plasmid-mediated *mcr-1* gene	4 or 8	4 or >4	No	+	No
69	*E. coli*	R	Plasmid-mediated *mcr-1* gene	64	>4	No	+	No
70	*E. coli*	R	Plasmid-mediated *mcr-2* gene	4	4	No	+	No
71	*K. oxytoca*	R	IS*Kpn26* into *mgrB* promotor	64	>4	No	+	No
72	*E. coli*	R	Unknown	8	**≤0.5**	**Yes, VME**	-	**Yes, VME**
73	*E. coli*	R	Unknown	8	>4	No	+	No
74	*E. coli*	R	Unknown	4	4	No	+	No
75	*E. coli*	R	Unknown	16	>4	No	+	No
76	*K. pneumoniae*	R	PmrA G53C	64	>4	No	+	No
77-78	*K. pneumoniae* (n=2)	R	PmrA G53S	16 or 32	>4	No	+	No
79-80	*K. pneumoniae* (n=2)	R	PmrB T157P	16 or 32	>4	No	+	No
81	*K. pneumoniae*	R	PhoP D191Y	128	**≤0.5**	**Yes, VME**	+	No
82	*K. pneumoniae*	R	PhoQ R16C	128	>4	No	+	No
83	*K. pneumoniae*	R	MgrB N42Y et K43I	64	>4	No	+	No
84	*K. pneumoniae*	R	MgrB I45T	64	>4	No	+	No
85 to 87	*K. pneumoniae* (n=3)	R	MgrB truncated	64 or 128	>4	No	+	No
88	*K. pneumoniae*	R	Deletion of 11 nucleotides into *mgrB* gene	>128	>4	No	+	No
89	*K. pneumoniae*	R	bla*CTX-M-15*/IS*Ecp1* into *mgrB*	64	>4	No	+	No
90	*K. pneumoniae*	R	IS*5* into *mgrB* gene	64	>4	No	+	No
91	*K. pneumoniae*	R	IS*102* into *mgrB* gene	>128	>4	No	+	No
92	*K. pneumoniae*	R	IS*Kpn14* into *mgrB* gene	32	>4	No	+	No
93	*K. pneumoniae*	R	IS*Kpn13* into *mgrB* gene	128	>4	No	+	No
94	*K. pneumoniae*	R	IS*Kpn26* into *mgrB* gene	64	>4	No	+	No
95	*K. pneumoniae*	R	IS*903* into *mgrB* gene	128	>4	No	+	No
96	*K. pneumoniae*	R	IS*903b* into *mgrB* gene	64	>4	No	+	No
97	*K. pneumoniae*	R	IS*5* into *mgrB* gene	128	>4	No	+	No
98	*K. pneumoniae*	R	IS*10R* into *mgrB* promotor	128	>4	No	+	No
99	*K. pneumoniae*	R	IS*Kpn14* into *mgrB* promotor	32	>4	No	+	No
100	*K. pneumoniae*	R	CrrB N141Y	>128	>4	No	+	No
101	*K. pneumoniae*	R	CrrB P151L	>128	>4	No	+	No
102	*K. pneumoniae*	R	CrrB G183V	>128	>4	No	+	No
103	*K. pneumoniae*	R	Plasmid mediated *mcr-1* gene	16	4	No	+	No
104	*K. pneumoniae*	R	Unknown	16	>4	No	+	No
105	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
106	*K. pneumoniae*	R	Unknown	32	>4	No	+	No
107	*K. pneumoniae*	R	Unknown	>128	>4	No	+	No
108	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
109	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
110	*K. pneumoniae*	R	Unknown	32	>4	No	+	No
111	*E. cloacae*	R	Unknown	32	>4	No	+	No
112	*E. cloacae*	R	Unknown	>128	>4	No	+	No
113	*E. cloacae*	R	Unknown	32	>4	No	+	No
114	*E. cloacae*	R	Unknown	>128	**1**	**Yes, VME**	+	No
115	*E. cloacae*	R	Unknown	64	**≤0.5**	**Yes, VME**	+	No
116	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
117	*E. cloacae*	R	Unknown	16	**≤0.5**	**Yes, VME**	+	No
118	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
119	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
120	*E. asburiae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
121	*S. enterica*	R	Plasmid mediated *mcr-1* gene	16	>4	No	+	No
122	*S. enterica*	R	Unknown	4	**2**	**Yes, VME**	+	No
123	*S. enterica*	R	Unknown	4	>4	No	+	No

Evaluation of a New Commercial Microarray Platform for the Simultaneous Detection of β-Lactamase and *mcr-1* and *mcr-2* Genes in *Enterobacteriaceae*

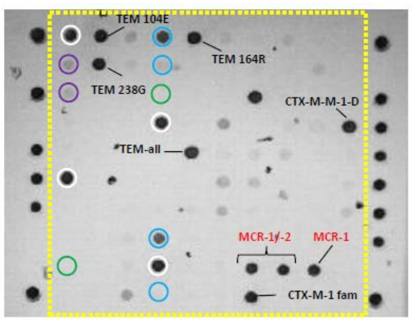
Journal of Clinical Microbiology October 2017 Volume 55 Issue 10

Odette J. Bernasconi,[a,b] Luigi Principe,[c] Regula Tinguely,[a] Aneta Karczmarek,[d] Vincent Perreten,[e] Francesco Luzzaro,[c] Andrea Endimiani[a]

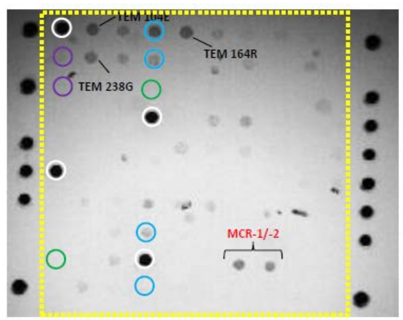
50-85 Euro

Species (no. of isolates)	No. (%) of strains											
	By origin:			Possessing relevant *bla* genes[c]:						Showing detection of *mcr-1* and *mcr-2* by:		
										Standard methods[e]		
	Human[b]	Animal	Food chain	None[g]	pAmpCs	ESBLs	pAmpCs and ESBLs	At least 1 carbapenemase	Colistin resistant[d]	*mcr-1*	*mcr-2*	New CT103XL microarray
E. coli (80)[a]	52 (65.0)	21 (26.3)	7 (8.8)	34 (42.5)	9 (11.2)	21 (26.2)	1 (1.2)	15 (18.7)	42 (52.5)	30 (37.5)	2 (2.5)	32[f] (100)
K. pneumoniae (14)	14 (100)	0	0	0	0	0	0	14 (100)	9 (64.3)	0	0	0
Other *Enterobacteriaceae* (12)[h]	11 (91.7)	0	1 (8.3)	7 (58.3)	0	2 (16.7)	0	3 (25.0)	10 (83.3)	0	0	0
Total (106)	77 (72.6)	21 (19.8)	8 (7.5)	41 (38.7)	9 (8.5)	23 (21.7)	1 (0.9)	32 (30.2)	61 (57.5)	30 (28.3)	2 (1.9)	32[f] (100)

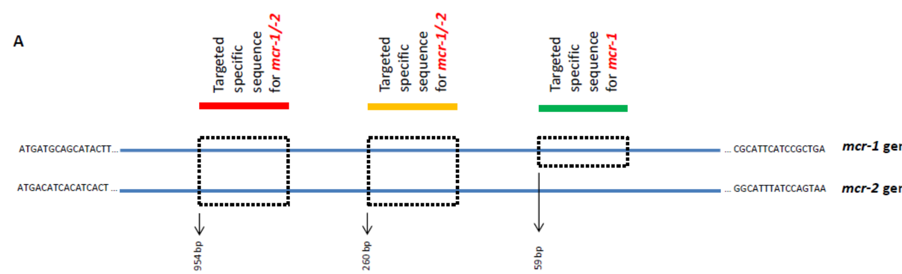
Highly accurate: both sens/spec: 100%



E. coli possessing *mcr-1*, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}1}$



E. coli possessing *mcr-2* and $bla_{TEM\text{-}1}$



Position of the ligation probes

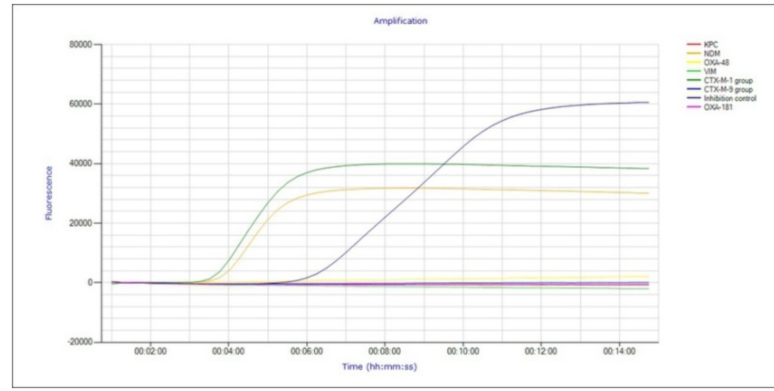
Evaluation of a Loop-Mediated Isothermal Amplification-Based Assay for the Rapid Detection of Plasmid-Encoded Colistin Resistance Gene *mcr-1* in *Enterobacteriaceae* Isolates

Antimicrobial Agents and Chemotherapy
April 2017 Volume 61 Issue 4

Genie II platform

~55 Euro

- Preparation (**2-5 min**)
- No DNA extraction
- Run time (**<30 min**)



LAMP (Eazyplex SuperBug *mcr-1* kit)
Loop-mediated isothermal Amplification
Real-time fluorescent measurement

104 *Enterobacteriaceae*:

- 67 mcr-1-positive
- 37 mcr-1-negative (including 9 colistin-R)

Sensitivity and Specificity: both 100%

TAT: < 20 minutes

A SYBR® Green-based real-time PCR method for improved detection of *mcr-1*-mediated colistin resistance in human stool samples

Valentina Donà[a], Odette J. Bernasconi[a,b], Sara Kasraian[a], Regula Tinguely[a], Andrea Endimiani[a,*]

Journal of Global Antimicrobial Resistance xxx (2017) xxx–xxx

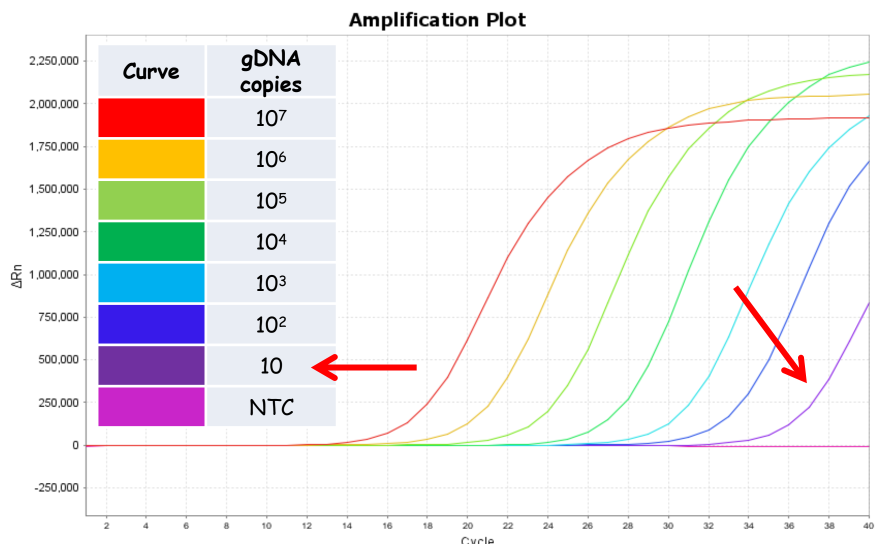
40 cycles with native stools

100 mg stools eluted in 100 µL
4 µL template; final reaction in 20 µL

HIV+ *E. coli* 31349 ST5 (IncX4)	Traveler *E. coli* #100 ST10 (IncHI2)	Traveler *E. coli* #19 ST5 (IncHI2)
Positive 37 cycles	Positive 34 cycles	Negative

30 cycles with the enrichment

HIV+ *E. coli* 31349 ST5 (IncX4)	Traveler *E. coli* #100 ST10 (IncHI2)	Traveler *E. coli* #19 ST5 (IncHI2)
Positive 21 cycles	Positive 23 cycles	Positive 22 cycles



LOD: 10 copies / reaction

Conclusions

The prevalence of COL-R *Enterobacteriaceae* is increasing

- Human hospital setting – especially *K. pneumoniae*
    - Chromosomal (mostly *mgrB*)
- Animal/food/environment settings – especially *E. coli*
    - Plasmid-mediated *mcr-1*-like based

Use of colistin

***mcr-1*-like are spreading rapidly**

- Plasmids highly stable and able to conjugate
- Transposition of the IS*AbI1* transposon
- Species carrying *mcr*-like genes

They can establish themselves into hyperepidemic MDR clones

Detection of COL-R strains (incl. *mcr-1*-like) presents issues

- BMD is not routinely implemented (Etest and disks not adequate)
- Automated systems have problems (VMEs)
- Not many molecular systems have been developed
    - Expensive
    - Most of them not for clinical samples

THANK YOU!

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